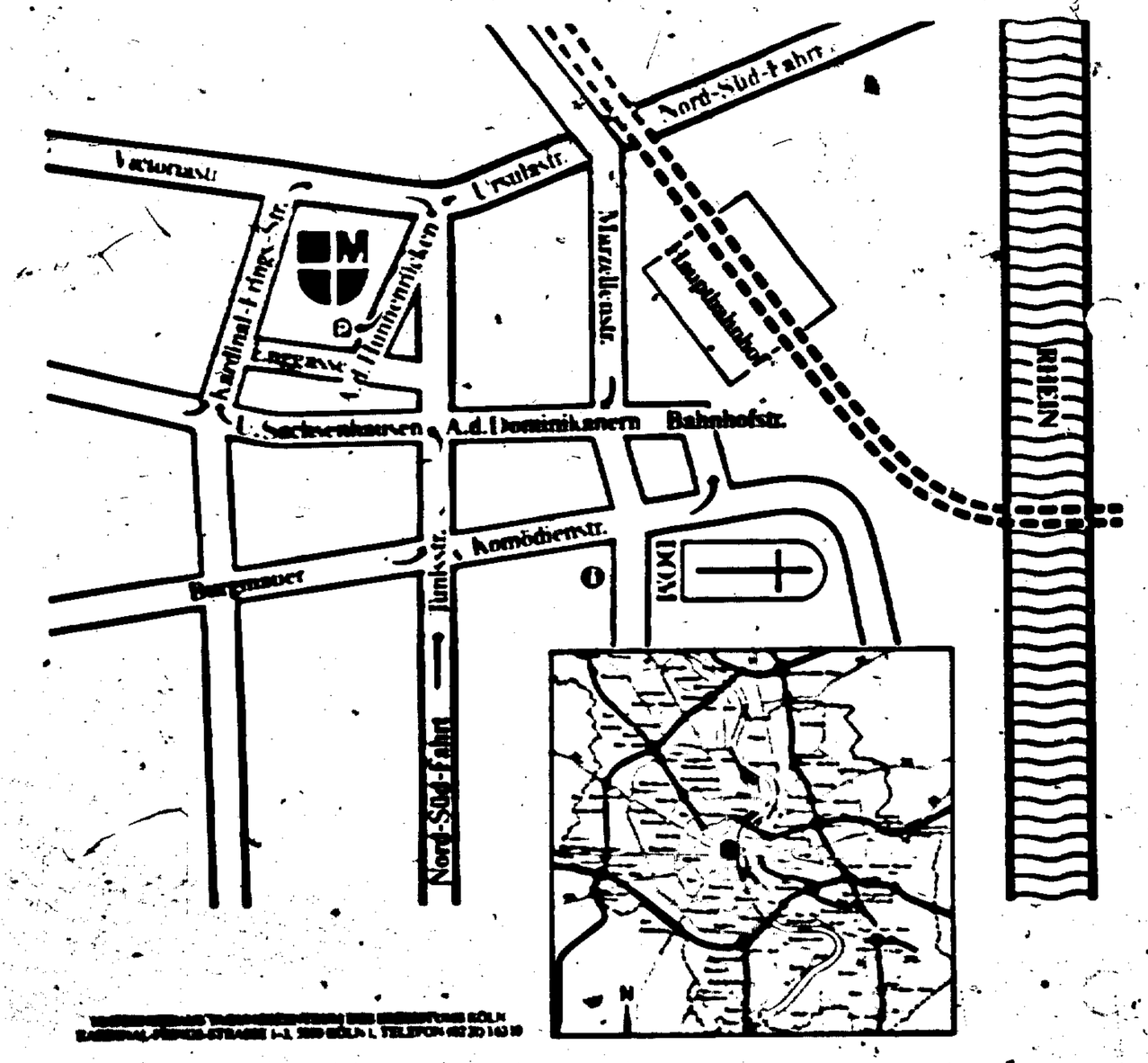
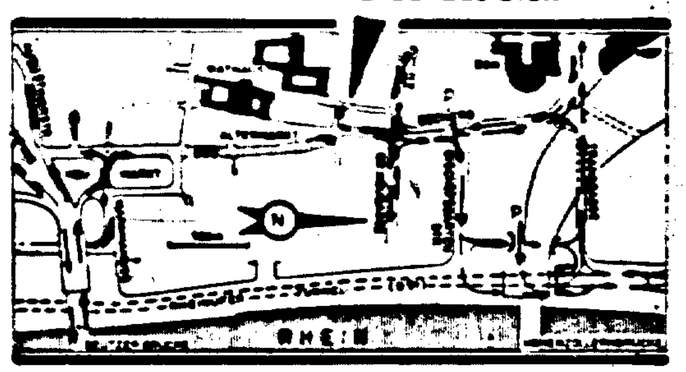



Brauhaus Sion




SPS SKIN PHARMACOLOGY SOCIETY

Fourth Annual Symposium
Cologne, West-Germany

Satellite Symposium of the XVII World Congress of Dermatology
Maternus-Haus
Sunday and Monday
May 31 st and June 1 st, 1987

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The goals of the society are:

1. To promote and encourage pharmacological research in the skin.
2. To introduce pharmacological knowledge and expertise into dermatological research.
3. To bring recent advances in skin pharmacology to the attention of general pharmacologists.
4. To encourage interest in the pathophysiology of the skin among general pharmacologists.
5. To increase standards of pharmacological research in skin.
6. To organize at least one scientific meeting annually and to initiate other scientific meetings as appropriate.

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## GENERATION OF EICOSANOIDS BY HUMAN SKIN MAST CELLS

Clive Robinson, R. Christopher Benyon and Martin K. Church, Clinical Pharmacology, Centre Block, Southampton General Hospital. Southampton SO9 4XY, U.K.

The ability of human skin mast cells to generate cyclooxygenase (CO) and lipoxygenase (LO) products of arachidonic acid was assessed in experiments in which cells dispersed from foreskin depleted or enriched up to 79% in mast cells by density gradient sedimentation were stimulated with calcium ionophore A23187. The profile of eicosanoids generated was examined in cells which had been incubated for 120 min with 250 uCi $^3$H-arachidonate. Cell preparations enriched to 79% mast cells generated $PGD_2$ as their primary CO produtt on stimulation with A23187. No $PGD_2$ was detected in mast cell depleted (0.15% mast cells) preparations. $PGE_2$ was detected in all preparations and $PGI_2$ (detected as 6-keto-$PGF_{1\alpha}$), 9α,11β-$PGF_2$ and, to a lesser extent, $PGF_{2\alpha}$, were seen in unfractionated and mast cell depleted preparations. $LTC_4$ was the major LO product seen in all preparations and the only one detected in preparations containing 79% mast cells. The association of $PGD_2$ and $LTC_4$ with mast cells and histamine release was confirmed by radioimmunoassay of unlabelled cell extracts. There was no correlation between other eicosanoids and mast cells. These results demonstrate the ability of human skin mast cells to generate $PGD_2$ and $LTC_4$ as their primary eicosanoid products and strongly suggest that they are the only source of these products in the cell dispersate.

## NON-IMMUNOLOGICAL STIMULATION OF HUMAN SKIN MAST CELLS

R. Christopher Benyon, Mark A. Lowman and Martin K. Church. Clinical Pharmacology, Centre Block, Southampton General Hospital, Southampton SO9 4XY, U.K.

Skin mast cells are unique amongst human mast cell populations so far studied in their ability to secrete histamine in response to activation by non-immunological stimuli including substance P (SP), morphine and compound 48/80. Like mast cells dispersed from human lung, adenoid, tonsil, intestinal mucosa and intestinal sub-mucosa, skin mast cells also respond to immunological (IgE-dependent) stimulation and calcium ionophore A23187. The mechanism of non-immunological stimulation differs from that of immunological in that it is faster (15 sec versus 6 min) and only partially dependent on extracellular calcium. However, secretion by both types of stimuli requires intact cellular pathways of glycolysis and oxidative phosphorylation. The weak activity of physalaemin, eledoisin and neurokinins A and B demonstrate that the SP activation site is distinct from the NK1, NK2 and NK3 receptors described in smooth muscle. The observation that the SP antagonist D-Pro$^4$,D-Trp$^{7,9,10}$$SP_{4-11}$ inhibits secretion induced by each of the non-immunological stimuli suggests that they activate a receptor of low specificity similar to that described in rat serosal mast cells.

Activation of human skin mast cells by a variety of peptides identified in dermal nerve-endings including SP and vasoactive intestinal polypeptide supports the hypothesis that mast cell products may mediate antidromic vasodilatation and neurogenic inflammation.

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## Information

Useful telephone numbers:

| | |
|---|---|
| Maternus Haus, Conference Center | 0221 - 16310 |
| Travel Agent (Hartmann) | 0221 - 7710446 |
| Hans F. Merk | 0221 - 478-8580 |

## Social events

| | | |
|---|---|---|
| Saturday May 30 st, | $19^{30}$ | Welcome Party |
| "Kölscher Abend" | | Sion-Bräu |
| Sunday May 31 st, | $20^{00}$ | Dinner |
| | $19^{30}$ | Bus transport from |
| | | Maternus Haus |
| | | Hotel Basler Hof |
| | | Excelsior Hotel Ernst |

Excursions (sight seeing Cologne), please contact registration office at Maternus Haus

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# PROGRAMME
## FOURTH ANNUAL SYMPOSIUM

**Saturday, May 30**

| | |
|---|---|
| 19.30 | Welcome Party "Kölscher Abend"<br>Sion-Bräu, Köln |

**Sunday, May 31**

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|---|---|
| 8.30 | Welcome G.K. Steigleder |
| Session 1: | Anticarcinogenesis<br>Chairman: David R. Bickers |
| 8.45- 9.05 | Anticarcinogenesis: Possibilities for modulation of initiation by modulation of metabolism<br>F. Oesch, Mainz |
| 9.10- 9.30 | Anticarcinogens as an approach for skin cancer prevention<br>H. Mukhtar, Cleveland |
| 9.35- 9.55 | Experimental multistage carcinogenesis: The Basis for the development of new concepts in cancer chemoprevention<br>F. Marks, Heidelberg |
| 10.00-10.20 | Anticarcinogenesis: Photoprotective role of Melanin<br>M.A. Pathak, Boston |
| 10.25-11.00 | Coffee break / Poster |
| Session 2: | Special lecture<br>Chairman: G.K. Steigleder |
| 11.00-11.30 | Cutaneous cytochrome P-450<br>David R. Bickers |

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**FLUCONAZOLE SKIN LEVELS DURING AND AFTER TREATMENT**

E. Haneke, Skin Hospital, D 5600 Wuppertal 1, FRG

Using shave biopsies, significant ketoconazole skin levels have recently been shown to persist in the skin after a 10-day treatment course. The study was performed to determine skin levels of fluconazole, a new orally active azole.

12 healthy volunteers were given 50 mg fluconazole daily for 14 days. Fluconazole levels in plasma, suction fluid and epidermis were determined by gas chromatography 2 hours after the first and last fluconazole intake as well as 5 (day 19) and 10 days (day 24) later.

Mean fluconazole plasma levels 2 hours after the first and last tablets as well as at days 19 and 24 were 1030, 2820, 275 and 21 ng/ml, respectively. Fluconazole concentrations in the blister fluid were 1192, 2965, 320 and 30.8 ng/ml, and epidermis levels were 5.55, 6.45, 3.55 and 1.91 ng/mg.

Fluconazole is well tolerated, water-soluble, mainly excreted with the urine and only a small portion is metabolized by the liver. Thus, its liver toxicity is expected to be lower than that of ketoconazole. Fluconazole skin concentrations after administration of 50 mg daily were found to be higher than ketoconazole skin levels after administration of 200 mg daily. For ketoconazole skin level measurements, however, shave biopsies containing a certain amount of papillary dermis have been used whereas pure epidermis was obtained by suction for fluconazole determinations. The study has shown that fluconazole and ketoconazole might be equally effective for short-term treatment of human skin mycoses.

**EFFECT OF CULTURE CONDITIONS ON METABOLISM OF PROPRANOLOL BY HUMAN KERATINOCYTES.**

Michel J. Cormier, Jean-Paul Marty, Department of Dermatology, Univ. of California School of Medicine, San Francisco, CA 94143, U.S.A. and Philip W. Ledger, ALZA Corporation, Palo Alto, CA 94303, U.S.A.

Little information exists on the ability of the epidermis to metabolize xenobiotic therapeutic agents. This study examines the metabolism of propranolol by human keratinocytes (HK) in vitro. In addition, manipulation of the [$Ca^{2+}$] of the culture medium has been used to induce differentiation (Boyce: J.Inv.Derm. 81:33s,1983) and concomitant effects of this on propranolol metabolism have been determined.

Adult and neonatal HK were cultured in MCDB 153 (0.1mM $Ca^{2+}$). At near confluency, passaged cells were induced to differentiate by pretreatment with 1.0mM $Ca^{2+}$ for 6, 2 and 0 days or at a range of [$Ca^{2+}$] for 2 days. Incubation was then continued in medium containing L-[4-3H]-propranolol ($10^{-7}$ to $10^{-4}$ M;10 µCi/ml) and $Ca^{2+}$ at pretreatment concentrations. Samples of medium (2, 4 and 6 days) and methanol extracts of cell layers (6 days) were analyzed for propranolol and metabolites by TLC. Release of metabolites into the medium was directly proportional to propranolol concentration and linear over 6 days. In 0.1mM $Ca^{2+}$ about 10% of the propranolol was metabolized during that time. Two major metabolites, naphthoxylactic acid (NLA) and propranolol glycol (GLY) and one minor, N-desisopropylpropranolol (DIP) were detected, both arising from side chain oxidation. No evidence was found for the formation of ring oxidation metabolites or conjugates. Pretreatment with 1.0mM $Ca^{2+}$ increased metabolites about twofold and raised the NLA/GLY ratio. Only very low levels of metabolites could be detected in extracts of cell layers.

Our results suggest that the state of keratinocyte differentiation can alter the xenobiotic metabolizing potential of those cells.

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| **Session 3:** | **Skin toxicology**<br>**Chairman: Helmut Appen** |
| 11.30-11.50 | Drug-induced experimental Porphyria: Animal models for human hepatic porphyrins<br>F. De Matteis, Carshalton |
| 11.55-12.05 | Toxicity of laser-activated hematoporphyrin derivates to cultured human epidermal keratinocytes<br>H. Kappus, Berlin |
| 12.10-12.20 | Chemical carcinogens as contact sensitizers in mice<br>C.A. Elmets, Cleveland |
| 12.25-12.85 | Current developments in diagnostic of drug allergies and pseudo-allergies<br>A.L. de Weck, Bern |
| 12.50-14.00 | Lunch |
| **Session 4:** | **Therapy**<br>**Chairman: M. Greaves** |
| 14.00-14.20 | Cyclosporine in psoriasis: Novel indications of known drugs<br>J.J. Voorhees, Ann Arbor |
| 14.25-14.35 | Antiproliferative and anticarcinogenic activities of immunosuppressive cyclosporins in mouse skin<br>F. Marks, Heidelberg |
| 14.40-14.50 | In vitro and in vivo anti-inflammatory activities of C10 substituted anthralin derivates<br>C. Hensby, Sophia Antipolis |
| 14.55-15.05 | The effects of isotretinoin on follicular and sebaceous gland differentiation<br>R. Marks, Cardiff |
| 15.10-15.20 | A new chemically stable radio-ligand (CD270) and assay procedure for cytosolic retinoic acid binding protein<br>J. Gazith, Sophia Antipolis |

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|---|---|
| 15.25-15.35 | Effects of lipoxygenase-derived eicosanoids on fibroblasts: Chemotactic activity and regulation of collagen synthesis<br>T. Ruzicka, München |
| 15.40-15.50 | Arachidonic acid induced ear oedema in three strains of rats and mice: A comparative study of anti-inflammatory drugs<br>M. Bouclier, Sophia Antipolis |
| 15.55-16.30 | Coffee break / Poster |
| Session 5: | Alternative methods I<br>Chairman: S. Yamamoto |
| 16.30-16.50 | Cell culture models in skin pharmacology and toxicology<br>U. Reichert, Sophia Antipolis |
| 16.55-17.05 | Use of air-exposed keratinocyte culture for pharmacological purposes<br>M. Ponec, Leiden |
| 17.10-17.20 | Comparative toxicity of antimicrobial agents on transformed human keratinocytes<br>F.M. Tetnall, London |
| 17.25-17.35 | Influence of dermal fibroblasts on epidermalization<br>B. Coulomb, Paris |
| Session 6: | Alternative methods II<br>Chairman: C. Hensby |
| 17.40-17.50 | 1,25 dihydroxyvitamin $D_3$ metabolism and receptor content in cultured human epidermal keratinocytes as a function of differentiation<br>P.M. Elias, San Francisco |
| 17.55-18.05 | A comparative study of the effects of NSAID's, corticosteroids, retinoids and anthralin on human PMN migration, oxidative burst, degranulation and 5-lipoxygenase activity<br>D. Cavey, Valbonne |

## THE EFFECT OF CALCIUM ON THE GRANULATION PROCESS

Roland Niedner Universitäts-Hautklinik Freiburg
D-7800 Freiburg, Hauptstr.7, Germany

Calcium plays a pivotal role in many important biological processes such as enzyme regulation, hormone and neurotransmitter release, muscle contraction and cell proliferation. The crucial, regulatory concentration of the free calcium ions in the cytoplasm is maintained and controlled by various active and passive calcium transport mechanisms located in the plasma membrane, in the endo-sarco-plasmic reticulum or in the mitochondria.
The influence of calcium on the proliferation of fibroblasts in vitro is well documented, but there are no studies in vivo. In an open woundgranulation model on guinea pigs calcium together with the calcium ionophor A 23 187 was given daily on the wound surface over a period of 10 days. Due to the calcium influx there was found a dose dependent augmentation of the woundgranulation. No effect could be demonstrated when calcium was given without the ionophor. At last a dose dependent reduction was seen when EGTA, a calcium chelator, and the ionophor were done on the wound. The ionophor enables the intracellular calcium to pass the cell membrane where it could be bound on EGTA, thus diminishing the intracellular content of free calcium. These results demonstrate the importance of calcium ions for the proliferation process in general, and the role of the intracellular calcium in particular.

## GUINEA PIG EPIDERMIS SYNTHESIZES 15-HYDROXY-8,11,13-EICOSATRIENOIC ACID (15-OH-20:3n6) FROM DIHOMOGAMMALINOLENIC ACID (DGLA): A POTENT LIPOXYGENASE INHIBITOR DERIVED FROM DIETARY PRIMROSE OIL.

Vincent A. Ziboh, Craig C. Miller, and A. Daniel Jones, Departments of Dermatology and Facility for Advanced Instrumentation, University of California, Davis, California.

Although primrose oil has been used in the management of cutaneous hyperproliferative disorders, the mechanism of its effect remains unknown. Prompted by a previous demonstration from our laboratory that gammalinolenic acid (GLA, 18:3n6), a constituent of primrose oil is elongated to DGLA (20:3n6) in both human and GP epidermis, we incubated GP epidermal microsomal preparations with $^{14}$C-20:3n6 (1.0 uCi). The radiolabelled metabolites were separated by high performance liquid chromatography (HPLC) into cyclooxygenase and lipoxygenase products. Further fractionation of the lipoxygenase products by HPLC revealed that the major $^{14}$C-metabolite was chromatographically similar to a 15-hydroxy-20:3n6 acid. The identity of the product was confirmed by gas chromatography/mass spectrometry. Since this hydroxy acid has been reported to inhibit 5-lipoxygenase activity in PMNS (cells known to infiltrate lesional psoriasis) we tested varying concentrations (5-50 uM) of 15-OH-20:3n6 on the conversion of $^{14}$C-20:4n6 (AA) into lipoxygenase products by GP epidermal enzyme preparations. Analysis of the radiolabelled metabolites showed that 15-OH-20:3n6 inhibited lipoxygenase activity in a dose-dependent fashion (inhibiting 55% at 50 uM). These data suggest that the ameliorative effects of primrose oil on hyperproliferative skin disorders may be due to the inhibitory effect of 15-OH-20:3n6 metabolite on epidermal lipoxygenase activity.

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## A NOVEL MOLECULAR EFFECT OF DITHRANOL AFFECTING GENE - EXPRESSION

A. Bernd, H. Holzmann, Universitäts-Hautklinik, Theodor - Stern-Kai 7, D-6000 Frankfurt/M., West Germany

The mode of action of dithranol is thought to be through its effect on DNA-replication, on repair synthesis, and on enzyme systems of polyamine synthesis and respiration. In a novel approach we investigated the influence of dithranol on the activity of nuclear envelope associated nucleoside triphosphatase (NTPase) in intact cell systems (primary human skin fibroblasts) and enzyme preparations (isolated from mouse fibroblasts 3T3).The NTPase is thought to be responsible for regulation of nucleo-cytoplasmic transport of mRNA. Dithranol was found to inhibit the NTPase efficiently in intact cells. Logarithmically growing human skin fibroblasts were incubated in the presence and the absence of 1µg/ml dithranol for 24 hrs. Subsequently the nuclear ghosts were prepared and the NTPase activity was determined. The level of NTPase activity was found to be 0.40 ± 0.029 µmol $P_i$/h x $10^8$ghosts. The enzyme activity in control cells were determined to be 3.3 ± 0.13 µmol $P_i$/h x $10^8$ ghosts. Addition of dithranol (1µg/ml to 10µg/ml) directly to isolated nuclear ghosts resulted also in a significant change in NTPase activity. The activities of nuclear envelope associated protein phosphokinase and protein phosphatase were identical in control as well as drug treated nuclear ghosts. These results suggest, that dithranol is obviously able to effect gene expression on the level of nuclear-cytoplasmic mRNA transport. The suppression of NTPase activity can be induced selectively in a direct manner.

## EVALUATION OF HUMAN EPIDERMAL PROLIFERATION AND DIFFERENTIATION IN VITRO BY FLOW CYTOMETRY ANALYSIS.

C. Lebreton[1], B. Coulomb[1], R. Migliorini[2], X. Renot[3], L. Dubertret[1] - (1) INSERM U 312 Dept. of Dermatology, Hôpital Henri Mondor, 94010 CRETEIL - (2) Dept. of Flow Cytometry, Hôp. St Louis, PARIS - (3) Lab. of cellular pharmacology, EPHE, PARIS - FRANCE.

We recently described a method of epidermalization in vitro that permits to obtain a well differentiated human epidermis and to quantify this epidermalization (Coulomb et all., Br. J. of Dermatol., 114, 91-101, 1986). In the present study we modulate culture conditions in order to modify the epidermal differentiation and to follow the response of the different criterion used to caracterize this differentiation, in particular DNA analysis with flow cytometry.

Human dermal fibroblasts are combined with collagen and culture medium. After polymerisation of the gel, 1 mm skin punch biopsy is implanted. The gel contracts into a tissue (The dermal equivalent: DE) within which the biopsy is tightly held in place. From this biopsy, an epidermis overgrew the surface of the DE. Three days after the implantation, the culture is surelevated or not on a stainless steel grid and the medium is replaced by fresh medium containing 20 ng/ml EGF completed or not with 0.4 µg/ml hydrocortisone and $10^{-10}$M cholera-toxin. The differentiation of the epidermis is investigated by morphology and by DNA content correlated with the growth area (DNA/S). The cell repartition in the different phases of the cell cycle is measured by DNA analysis with flow cytometry after dissociation of the epidermis by trypsin and mithramycin coloration. Epidermis from suction blister roof were used as control.

The complete immersion of the culture prevents the good differentiation observed when epidermis is in contact with air. In particular, no cornified layer is formed. This difference is also evaluated by the criterion DNA/S (Emersion : 0.15 µg/mm$^2$ Immersion : 0.05 µg/mm$^2$). Nevertheless, even in emerged conditions, this DNA/S is inferior to the in vivo one (0.3 µg/mm$^2$). The suppression of hydrocortisone and cholera-toxin from the medium do not significatively modified the morphology or the DNA/S, either in emerged or immersed conditions.

The flow cytometry analysis confirms and complete these results. This technics allows to recognize the proportion of the DNA corresponding to the S and G2 phases, and so to evaluate the cell divisions. When compared with epidermis in vivo, growth rate is equal in emerged conditions when all the factors are presents, or higher when EGF is alone, while it decreases in immersion.

Thus, flow cytometry, that is usualy used for cell cycle analysis of homogeneous cell populations, or for cell sorting, is an important tool for epidermal proliferation and differentiation evaluation.

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| 18.10-18.30 | Improvement and validation of in-vitro methods for routine testing in dermato-toxicology<br>N.-P. Lüpke, Münster |
| 18.35 | End of scientific session |
| 20.00 | Dinner<br>19.30 bus transport: Maternus Haus<br>Hotel Basler Hof<br>Excelsior Hotel Ernst |

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Monday, June 1

**Session 7:** **Therapy II**
**Chairman: G. Stüttgen**

| | |
|---|---|
| 8.30- 8.50 | Interferons in dermatology<br>C. Mahrle, Köln |
| 8.55- 9.08 | A new method to study pruritus and anti-pruritic drugs<br>C.F. Wahlgren, Stockholm |
| 9.10-9.30 | Calcium channel antagonists in dermatology<br>Novel indications of known drugs<br>Pauline Dowd, London |
| 9.35- 9.45 | Effect of cimetidine on murine allergic contact hypersensitivity (ACH)<br>N.A. Soter, New York |
| 9.50-10.00 | The influence of $Ca^{++}$ modulators on epidermal cells in vitro. A protective effect from $Ca^{++}$ overload?<br>B. Thiele, Köln |
| 10.05-10.25 | Serotonin antagonists: novel indications of known drugs<br>R. Verheughe, Leuven |
| 10.30-11.00 | Coffee break / Poster |

**Session 8:** **Therapy III**
**Chairman: Hans Schaefer**

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|---|---|
| 11.00-11.20 | Cytochrome P-450 isoenzymes: Target for azole derivatives<br>H. Vanden Bossche, Beerse |
| 11.25-11.35 | Protection against chemically induced skin tumorigenesis by naturally occurring plant phenols in sencar mice<br>M. Das, Lucknow |

## INVOLVEMENT OF LIPOXYGENASE METABOLITES OF ARACHIDONATE AND PLATELET ACTIVATING FACTOR IN MURINE ALLERGIC AND IRRITANT CONTACT DERMATITIS

M. Csató, T. Rosenbach and B.M. Czarnetzki

Department of Dermatology, University of Münster, D-4400 Münster, Federal Republic of Germany

We have recently demonstrated that a specific peptidoleukotriene receptor antagonist is able to suppress the early stages of murine experimental contact dermatitis. Further potential candidates for mediating cutaneous inflammation are other arachidonate metabolites and platelet activating factor (PAF), a highly potent phospholipid agent with pro-inflammatory and chemotactic activities. We have therefore investigated the effects of locally applied inhibitors for the phospholipase $A_2$-arachidonate-5-lipoxygenase pathway and of antagonists for PAF in experimental allergic and irritant contact dermatitis in the mouse. Dinitrofluorobenzene-induced allergic and croton oil-induced irritant contact dermatitis was evaluated by measuring the ear swelling in groups of six animals in 5 subsequent days. Both the inhibitors of the 5-lipoxygenase pathways and the antagonists for PAF were applied directly after elucidation of the dermatitis. PAF and 5-lipoxygenase inhibitors were more active than phospholipase $A_2$ inhibitors. Reduction of ear swelling was optimal during the first two to three days after elicitation. The supression was more prominent in the allergic than in the irritant dermatitis.
Our results give supportive evidence for the involvement of the 5-lipoxygenase metabolites of arachidonate and of PAF in the early stages of murine contact dermatitis of the allergic and less so the irritant type.

## DEXAMETHASONE INDUCES ENHANCEMENT OF PROSTAGLANDIN $D_2$ SYNTHESIS AND APPEARANCE OF ENDOGENOUS PEROXIDASE IN BONE-MARROW DERIVED MAST CELLS.

MICHEL L.°, PITTON C°., PROST C.°, MENCIA-HUERTA J.M.° BENVENISTE J.° and DUBERTRET L.° . °INSERM U 312, Hôpital Henri Mondor, 94010 Créteil-France. °INSERM U 200, 32 rue des Carnets, 92140 Clamart-France.

Mouse bone marrow derived mast cells (BMMC), obtained from culture of bone marrow cells in presence of concavalin A-conditioned medium, have been shown to be functionally and ultrastructurally analogous to mucosal mast cells. After immunological stimulation, both cell types generate very low amounts of prostaglandin (PG) $D_2$. Addition of 1 µM dexamethasone (DM) for 24 hr to BMMC culture medium enhances $PGD_2$ synthesis in BMMC.

Endogenous peroxidase, an enzyme destroyed by cell fixation, has been associated with PG synthesis in human platelets and monocytes. Peroxidase has been localized in the perinuclear envelope (NE) and in the endoplasmic reticulum (ER) of human monocytes and connective tissue mast cells.
We studied the immunologically-induced synthesis of $PGD_2$ and the ultrastructural presence of peroxidase in BMMC treated with 1 µM DM for 24 hr and up to 14 days.
After IgE sensitization and specific antigen stimulation, BMMC treated with DM for 24 hr and 14 days released respectively 2.6 and 7.6 more $PGD_2$ than untreated cells, to reach $58.03 \pm 6.15$ ng/$10^6$ cells after 14 days of DM treatment.

Using 3,3 diaminobenzidine (DAB) reaction prior to cell fixation, peroxidase was localized in NE of 24 hr-treated BMMC and in both NE and ER of 14 day-treated BMMC, whereas no peroxidase was detected in untreated cells. When cell fixation was carried out prior DAB reaction, no peroxidase was localized in NE or ER of both treated and untreated BMMC.

In conclusion, short- and long-term treatment of BMMC with DM induced the enhancement of immunologically-induced release of $PGD_2$ and the appearance of peroxidase localized in NE and ER. A change in the phenotype of BMMC toward that of mature connective tissue-type mast cells might occur under DM treatment. Moreover, our results suggest that endogenous peroxidase, destroyed by cell fixation, could be involved in $PGD_2$ synthesis in DM-treated BMMC.

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**BULLOUS ERYTHEMA MULTIFORME-LIKE BULLOUS PEMPHIGOID INDUCED BY VARIOUS PENICILLINS.**
J. Alcalay, M. David, A. Ingber, B. Hazat, M. Sandbank. Department of Dermatology, Beilinson Medical Center, Petah Tiqva, Israel.

In recent years, bullous pemphigoid (BP) has been reported to occur following treatment with several drugs. Two young adult males (aged 16 and 23) and a 50-year-old woman developed a severe bullous eruption shortly after taking procaine-penicillin G, amoxycillin and phenoxymethylpenicillin respectively. The patients had high fever, prostration and multiple erosions of the buccal mucosae, conjunctiva and the genitalia. Multiple large tense bullae were scattered on the faces and extremities of the patients and target-like erythematous lesions were noted on the palms and soles. The clinical diagnosis was bullous erythema multiforme in two patients and Stevens-Johnson syndrome in the third. Direct immunofluorescent study revealed in the patients a strong linear deposition of IgG and $C_3$ along the dermoepidermal junction. In the serum, IgG antibodies against the basement membrane were found in titers of 1:40, 1:80 and 1:320, respectively. The histological findings revealed subepidermal bullae. However, two of the patients had necrotic keratinocytes or thrombi in the dermal blood vessels. Complete recovery was achieved after treatment with prednisone. No scar formation or recurrence of the eruption were noted. The clinical and histological findings of our patients differ from those of classic BP and also from previously described drug-induced BP. We conclude that penicillins can cause a life-threatening BP-like eruption and that immunofluorescent findings cannot serve as a single criterion for determination of BP.

**ASSESSMENT OF THE DURATION OF ACTION OF TERFENADINE ON HISTAMINE INDUCED WEALS.** L. Shall and R. Marks, Department of Medicine, University of Wales College of Medicine, Cardiff.

Terfenadine is a selective H1-receptor antagonist which inhibits the skin reaction to intradermally injected histamine within two hours. Using a single dose of 20, 60 or 200 mg terfenadine, a dose dependant decrease in histamine weal area is produced which reaches a maximum by four hours. With the 60 and 200 mg doses, significant reduction of weal area persisted for eight hours. We aimed to quantify the reduction in weal area and thickness from 12 to 24 hours following a single dose of terfenadine. Ten healthy volunteers were given 120 mg of terfenadine or placebo in a double-blind randomised crossover study. 20µg of histamine was then injected intradermally at 12, 18 and 24 hrs. The thickness of the resulting weal was measured by an A-scan pulsed ultrasound device. The area of the weal and flare was measured by tracing onto acetate sheets and using a digitising tablet linked to a microcomputer. Before each injection, blood was taken for terfenadine levels. The results showed a statistically significant difference between terfenadine 120mg and placebo for weal area ($p<0.01$) and weal and flare area ($p<0.01$) but not for weal thickness (two-way analysis of variance). No difference in the degree of suppression of the wealing response could be demonstrated at the three time points chosen. Thus terfenadine suppresses the wealing response caused by intradermally injected histamine over a 24 hr period. It may therefore be possible to use a once daily dose of terfenadine.

| Time | Medication | Mean Area (mm²) (± SD) Weal | Weal & Flare |
|---|---|---|---|
| 12 hours | Terfenadine | 93.55 ± 32.89 | 1007 ± 516 |
| | Placebo | 187.18 ± 78.78 | 3030 ± 2111 |
| 18 hours | Terfenadine | 151.10 ±113.29 | 1068 ± 748 |
| | Placebo | 204.06 ± 72.34 | 2408 ± 1314 |
| 24 hours | Terfenadine | 138.87 ± 42.18 | 1452 ± 676 |
| | Placebo | 226.14 ±111.19 | 2580 ± 1510 |

| | |
|---|---|
| 11.40-11.50 | Inhibition of neutrophil chemotactic factor release from guinea pig skin by dexamethasone in anaphylactic reaction<br>T. Numata, Hiroshima |
| 11.55-12.05 | Transcutaneous collection of systemically administered theophylline in rhesus monkeys<br>D.P. Conner, Bethesda |
| 12.10-12.30 | Antiandrogens: novel indications of known drugs<br>H. Wendt, Berlin |
| 12.35 | Poster Award |
| 12.40 | Closing remarks |
| 12.45 | End of the meeting |

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**Posters**

J.R. Gibson, J.E. Hough, P. Marks, A. Webster
Extemporaneous and proprietary dilution of corticosteroid ointments - practical considerations concerning potency

B. Lambrey, W. Schalla, M.H. Daniel, N. Kali, H. Schaefer
Skin permeability using hydrocortisone lotion in two dermatosis models

C.N. Hensby, C. Delescluse, P. Vaigot, J. Bailly
The effects of anti-inflammatory agents on oedema and DNA synthesis induced by 12-0-tetradecanoylphorbol-13-acetate in the guinea pig

A. Chatelus, J. Ferracin, C. Delain, C. Hensby
The "rhino mouse" model for the assay of comedolytic activity of agents used in the treatment of acne

M. Bouclier, A. Jomard, J. Eustache, N. Kali, B. Shroot, C. Hensby
Control of animal variables in the tape stripping model of ornithine decarboxylase induction in hairless rat

G. Siegenthaler, J. Gazith, B. Shroot, J. Eustache, O. Watts, J.H. Saurat
Quantitation of CRABP in human epidermis. Comparison of results using retinoic acid and a stable retinoid (CD270)

J.M. Bernadon, M. Bouclier, M.T. Cavey, C. Delescluse, J. Eustache, J. Gazith, B. Shroot
Heterocyclic retinoids: A new series of stable molecules with retinoid like activity

W. Schalla, L. Dutail
Non-invasive assessment of an antipsoriatic therapy (PUVA)

E. Kohen, J.P. Reyftmann, P. Morliere, R. Santus, C. Kohen, W.F. Mangel, L. Dubertret, J.G. Hirschberg
Microspectrofluorometric study of porphyrin photosensitization on simple living cells

MICROSPECTROFLUOROMETRIC STUDY OF PORPHYRIN PHOTOSENSITIZATION ON SIMPLE LIVING CELLS.

E. KOHEN[1], J.P. REYFTMANN[2], P. MORLIERE[3], R. SANTUS[2], C. KOHEN[1], W.F. MANGEL[4], L. DUBERTRET[3] and J.G. HIRSCHBERG[5]
[1]University of Miami, Department of Biology, POB 249,118 Coral Gables, FL 33124, USA; [2]Museum National d'Histoire Naturelle, Laboratoire de Physico-Chimie de l'Adaptation Biologique, CNRS-UA 481, 43, rue Cuvier, 75231, Paris Cedex, France; [3]Hôpital Henri Mondor, INSERM U 312, Laboratoire de Dermatologie, 94010 Créteil, France.; [4]Brookhaven National Laboratory, Biology Department, Upton, NY 11973, USA and [5]University of Miami, Department of Physics, POB 248,046 Coral Gables, FL 33124, USA.

The microspectrofluorometric technique was used to investigate the photosensitization of single living cell (L cell fibroblasts) by either lipophilic or hydrophobic porphyrins. The formation of fluorescent lipofuscin-like pigments ($\lambda$max=450 nm) implying lipid peroxidation is shown to be a primary effect of the porphyrin photosensitization of membranes. These fluorescent products are also formed in the dark when cells are preirradiated in the presence of the photosensitizer. Membrane alterations are also demonstrated at the level of lysosomes.

Permeation of lysosomal membranes is illustrated using fluorogenic substrates for β-galactosidase or peptidase. This permeation as well as lipofuscin like pigments formation are prevented or retarded by polyenes such as crocetin or tigason. However the lysosomal membrane permeation is not inhibited by chloroquine, a lysosomotropic drug. Metabolic alterations photosensitized by porphyrin were also observed. The microinjection of malate or 6-phosphogluconate to photosensitized cells demonstrate that both Kreb's cycle and pentose pathway are altered, as shown by the transient charges in the NAD(P)H fluorescence following the injection.

IN VITRO PHOTOSENSITIZING PROPERTIES OF KHELLIN

P. MORLIERE*, G. HUPPE*, R. SANTUS°, and L. DUBERTRET*, *INSERM U. 312, Dermatology, Hopital Henri Mondor, 94010, Créteil-France - °Museum National d'Histoire Naturelle, Laboratoire de Physico-Chimie de l'Adaptation Biologique, CNRS-UA 481, 43, rue Cuvier, 75231 Paris-Cedex-France.

Khellin, a current coronary vasodilator, exhibits beneficial photosensitizing properties at the skin level, in treating vitiligo for example. Its chemical structure closely resembles the psoralens structure and therefore one might suspect khellin to exhibit some in vitro photosensitizing properties of psoralens.

In aqueous solution, khellin is much more soluble than most psoralens but exhibits lower absorption properties. In aqueous medium, UVA maximum is found at 337 nm with a molar extinction coefficient of about 4200 $M^{-1}cm^{-1}$.

Equilibrium dialyses show that khellin complexes with DNA with an affinity constant of 2000 $M^{-1}$ and 3 binding sites per 100 nucleotides, which is lower than that found for 5-Methoxypsoralen for example.

Crosslinking to DNA was investigated by measuring the percentages of non renaturating fraction in irradiated DNA khellin complexes or irradiated DNA-5-methoxypsoralen complexes with respect to DNA alone. The results clearly show that khellin does not behave as a bifunctional agent, in contrast to what is observed for 5-Methoxypsoralen.

Type II and type I photodynamic properties were investigated with His and Trp as substrates respectively. The His photosensitized oxidation quantum yield is about $8x10^{-5}$ while it raises $1x10^{-3}$, $4x10^{-3}$ and $1.8x10^{-2}$ and for 5-Methoxy, 8-Methoxy and psoralen respectively.

This illustrates that khellin is a poor type II photosensitizer. The Trp photosensitized oxidation quantum yield is about $1x10^{-4}$ which is low compared to the one obtained with 5-Methoxypsoralen as photosensitizer, e.g. $5x10^{-3}$. Khellin is therefore a poor type I photosensitizer too.

In conclusion, in view of its skin photosensitizing properties and its very poor in vitro photosensitizing properties, khellin appears to be a very interesting chromophore. It may be thought to offer peculiar mechanisms for its skin photosensitizing action.

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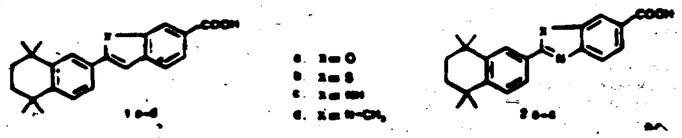
## HETEROCYCLIC RETINOIDS: A NEW SERIES OF STABLE MOLECULES WITH RETINOID LIKE ACTIVITY

J.M.Bernardon, M.Boucher, M.T.Cavey, C.Delescluse, J.Eustache, J.Gazith and B.Shroot
*Centre International de Recherches Dermatologiques (CIRD), Sophia-Antipolis, 06565 Valbonne-Cedex, France*

The discovery of the "arotinoids" has been an important milestone in retinoid research, leading to molecules which are reported to have increased biological activities and significantly improved therapeutic indexes. Subsequently the constrained arotinoid analog 6-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)-2-naphthoic acid (TTNN), the first representative of a new family of stable fully aromatic retinoids was described. Although TTNN was found to be a potent retinoid, its activity did not approach that of arotinoid Ro-13-7410. We hypothesized that this unexpected result could be in part due to the different nature of the double bond corresponding to that between $C_8$ and $C_{10}$ in retinoic acid: aromatic in TTNN, olefinic in arotinoid. We now describe a series of new heterocyclic retinoids of general structures 1 and 2, in which the aromatic character of the appropriate double bond is modulated.




The compounds were evaluated in two models: *in-vivo* inhibition of induced ornithine decarboxylase activity in rat skin (ODC), and *in-vitro* induction of differentiation of mouse embryo teratocarcinoma cell line F9 (F 9). Binding to cRABP was also measured. Depending on the nature of the heterocyclic system, striking differences in the corresponding biological activities were observed. The most potent molecules were the two sulfur containing compounds 1b and 2b, and the benzothiophene 1b is more active than TTNN in these tests. The compounds 1a, 2c, 1c, 1d, and 2a then showed gradually decreasing but still very significant activities approaching that of retinoic acid. Binding to cRABP could not be strictly correlated with the results obtained in the F9 and ODC assays.

## NON-INVASIVE ASSESSMENT OF AN ANTIPSORIATIC THERAPY (PUVA)

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CENTRE INTERNATIONAL DE RECHERCHES DERMATOLOGIQUES (CIRD)
SOPHIA ANTIPOLIS, F-06565 VALBONE CEDEX FRANCE

Some effects of an antipsoriatic treatment have been investigated using non-invasive measurements on involved and uninvolved skin of psoriatic patients (n=17). The patients were divided into two groups according to the location of measurements, which where done either on the forearm (n=10) or on the lower limbs (n=7). Skin blood flow (SBF) was measured by the Laser Doppler technique (PERIFLUX$^R$, Perimed, Stockholm), transcutaneous oxygen pressure ($tcpO_2$) by steady state gas diffusion (TCM2, Radiometer$^R$ Copenhagen), and transepidermal water loss (TEWL) by gradient humidity detection (Evaporimeter 1P, Servomed, Stockholm). Each parameter was studied as an absolute value and was also calculated as the ratio of the value of involved to uninvolved skin in order to eliminate physiological variations over time. In addition, visual assessments were graded using a score system (1-4).

On the forearm, the parameters returned to uninvolved level during the treatment in a non-linear fashion. SBF needed more time than the others to reach the level of uninvolved skin. The $tcpO_2$ at 37°C was correlated to TEWL as measurement of skin permeability. Both decreased rapidly in the first three weeks of treatment in a linear manner and somewhat slower thereafter. The $tcpO_2$ at 44°C starting from a decreased ratio behaved differently. After a plateau until the 9-11$^{th}$ session, it returned abruptly to uninvolved value. The low values of $tcpO_2$ on psoriatic plaques could be explained by two main assumptions: either by an increase of $O_2$ consumption or by a diminution in blood flow in lesional skin leading to a larger arterio-venous difference in $pO_2$. For that purpose we have undertaken SBF measurements at 44°C. The results show that SBF is always higher on the lesions and thereby confirm the first assumption. Good correlations ($r > 0.9$) were found between the parameters and the visual assessments, except with $tcpO_2$ at 44°C. On the legs, an identical evolution could be observed for all parameters. The data were more dispersed (greater SEM) and the ratio did not reach unity within the same treatment period. This fact was probably due to the longer time needed to obtain remissions on psoriatic lesions on lower limbs.



P. Morliere, G. Huppe, R. Santus, L. Dubertret
In vitro photosensitizing properties of khellin

J. Alcalay, M. David, A. Ingber, B. Hazaz, M. Sandbank
Bullous erythema multiforme-like bullous pemphigoid induced by various penicillins

L. Shall, R. Marks
Assessment of the duration of action of terfenadine on histamine induced weals

L. Michel, C. Pitton, C. Prost, J.M. Mencia-Huerta, J. Benveniste, L. Dubertret
Dexamethasone induces enhancement of prostaglandin $D_2$ synthesis and appearance of endogenous peroxidase in bone-marrow derived mast cells

M. Csató, T. Rosenbach, B.M. Czarnetzki
Involvement of lipoxygenase metabolites of arachidonate and platelet activating factor in murine allergic and irritant contact dermatitis

A. Bernd, H. Holzmann
A novel molecular effect of dithranol affecting gene - expression

C. Lebreton, B. Coulomb, R. Miglierina, X. Ronot, L. Dubertret
Evaluation of human epidermal proliferation and differentiation in vitro by flow cytometry analysis

R. Niedner
The effect of calcium on the granulation process

V.A. Ziboh, C.C. Miller, A.D. Jones
Guinea pig epidermis synthesizes 15-hydroxy-8,11,13-Eicosatrienoic acid (15-OH-20:3n6) from dihomogammalinolenic acid (DGLA): A potent lipoxygenase inhibitor derived from dietary primose oil

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M.J. Cormier, J.P. Marty
Effect of culture conditions on metabolism of propranolol by human keratinocytes

E. Heneke
Fluconazole skin levels during and after treatment

S. Ivankovic, S. Kempf, F.-W. Kühne, K.-W. Stahl
Dermal rescue therapy with TCDO after whole body γ-irradiation

CONTROL OF ANIMAL VARIABLES IN THE TAPE STRIPPING MODEL OF ORNITHINE DECARBOXYLASE INDUCTION IN HAIRLESS RAT.

M. Bouclier, A. Jomard, J. Eustache, N. Kail, B. Shroot and C. Hensby. Centre International de Recherches Dermatologiques, Sophia-Antipolis, 06560 VALBONNE, France.

In mouse skin, anti-proliferative agents including retinoids and anthralin inhibit induction of ornithine decarboxylase activity by a variety of hyperproliferative stimuli. Recently we published the assay of ornithine decarboxylase in the hairless rat epidermis after tape stripping for a rapid evaluation of new retinoids (J. Pharm. Method. 16, 151-160 1986). In order to minimize experimental variability introduced by the animal in our model we examined a number of animal parameters using a single lot of 50 males and 50 females aged from 4 to 11 weeks. Hair development was more pronounced in males, with two "regrowth" periods observed in both sexes, the first period at 6-7 weeks and the second period at 10-11 weeks. Stratum corneum thickness was not significantly changed either between males and females or as a function of age. Stripping efficiency was best in females aged 7-11 weeks. Induction of ornithine decarboxylase activity in the epidermis was much more reproducible inter animal in females. Results obtained with retinoic acid in a large number of subsequent tests using female rats aged 8 weeks have given improved and very reproducible results from one experiment to another. Our results show that animal models need to be well standardised to permit valid pharmacological comparisons.

QUANTITATION OF CRABP IN HUMAN EPIDERMIS - COMPARISON OF RESULTS USING RETINOIC ACID AND A STABLE RETINOID (CD270).

G. Siegenthaler[1], J. Gazith[1], B. Shroot[2], J. Eustache[2], O. Watts[2] and J.H. Saurat[1]. [1]Clinique de Dermatologie, Hopital cantonal Universitaire, 1211 Geneve-4, Switzerland and [2]Centre International de Recherches Dermatologiques (CIRD), Sophia Antipolis, Valbonne, France.

The cytosolic retinoic acid binding protein (cRABP) is assumed to mediate (at least in part) the biological or therapeutical activity of retinoic acid (RA). The importance of this binding protein is further emphasized by the fact that cRABP concentration has been shown to be altered in retinoid-responsive skin diseases and in cases of abnormal differentiation. There is evidence to suggest that the amount of cRABP in the skin can be elevated by retinoid therapy (up-regulation).
The amount of cRABP is usually quantitated by binding assay. RA has been used as the radioactive ligand, though it is sensitive to light and oxidation and its use entails the employment of special precautions such as the inclusion of anti-oxidants, working in dimmed or actinic light and storage under an inert atmosphere.
Recently, a new retinoid has been proposed as a RA substitute for binding to cRABP. The new retinoid (CD270) is a substituted benzo-[b]-thiophenecarboxylic acid derivative, was developed at CIRD and is available now as a tritiated derivative. CD270 was shown to bind to cRABP and to have affinity and specificity similar to that of retinoic acid. This molecule, which contains no olefinic double bonds, is chemically stable and is unaffected by light or atmospheric oxidation.
Using PAGE and charcoal-dextran techniques, we have shown that CD270 binds specifically to cRABP from human epidermis. A higher level of cRABP was measured with [$^3$H]-CD270 (13+/- 4 pmol/mg protein: n=5) as compared with [$^3$H]-RA (5.6 +/- 2.2 pmol/mg protein: n=5). The $IC_{50}$ of CD270 was found to be identical to that of RA. PAGE-immunoblotting shows that CD270 has no affinity for plasma retinol binding protein (RBP). The latter may be present as a contaminant in skin homogenates, and binds retinoic acid to a considerable extent.
These results suggest that CD270 binds to cRABP with the same affinity as RA. Unlike RA, however, it does not bind to RBP and is easier to handle then RA.

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THE EFFECTS OF ANTI-INFLAMMATORY AGENTS ON OEDEMA AND DNA SYNTHESIS INDUCED BY 12-O-TETRADECANOYLPHORBOL-13-ACETATE IN THE GUINEA PIG.

C.N. Hensby[1], C. Delescluse[2], P. Valgot[2], and J. Bailly[2],
[1] Department of Experimental Pharmacology and Toxicology,
[2] Cell Biology Department, Centre International de Recherches Dermatologiques (CIRD), Sophia Antipolis, F-06565 Valbonne, FRANCE.

Inflammation and hyperplasia are frequently associated in skin diseases. In order to verify this relationship, we studied the antagonistic effect of different classes of anti-inflammatory agents on the inflammatory and hyperplasiogenic responses elicited by one topical application of 12-O-Tetradecanoylphorbol-13-acetate (TPA) to the ear of the guinea-pig. Oedema and DNA synthesis were chosen as relevant parameters.

Hydrocortisone, betamethazone-17-valerate, quinacrine, ETYA, indomethacin, and phenylbutazone were applied topically The oedema was estimated according to time by the ratio ear weight/ear surface. At 24 hours after TPA treatment, the number of cells in $G_1$, S and $G_2$ phases was measured by flowcytophotometry. The percentage of cells in $G_1$, S and $G_2$ phases was estimated by planimetry.

All anti-inflammatory agents tested significantly inhibited DNA synthesis induced by TPA. Moreover, all compounds except quinacrine and phenylbutazone also inhibited oedema formation. In conclusion, our results demonstrate that while oedema and hyperplasia are frequently associated, this is not always the case.

THE "RHINO MOUSE" MODEL FOR THE ASSAY OF COMEDOLYTIC ACTIVITY OF AGENTS USED IN THE TREATMENT OF ACNE.

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Centre International de Recherches Dermatologiques (CIRD)
Sophia-Antipolis. 06565 Valbonne, France.

Several experimental models including the rhino mouse have been developed for the screening of topically active comedolytic agents. The rhino mouse ($hr^{hr}$ $hr^{hr}$) is allelic to the more familiar smooth skinned hairless (hr hr) variety and whose skin presents a high density of spontaneous comedones which appear of histological sections as cystic formations.

To standardize this model, we have performed dose response curves with topical all-trans-retinoic acid and benzoyl peroxide. Test agents were applied to the dorsal trunk once daily, on a 5 day/week basis for 3 weeks. Skin biopsies obtained at 1, 2 and 3 weeks, were sectionned (3 um), fixed and stained with HPS (Hematoxylin, Phloxyn and Safran) for examination by light microscopy. From each biopsie 3 sections were cut at a 150 um intervals. Image Analysis was performed with a KONTRON MOP- VIDEOPLAN linked to a digital table and TV monitor. Various parameters were analysed including the number of open and epidermic comedones, the "Comedone Profile" described by C.BONNE (Int.J.Cosm.Sci.1981, 3, 23-28), the number of dermic cysts and the variation of epidermal thickness. Treatment with all-trans-retinoic acid (0.3 ; 0.1 ; 0.03 and 0.01 %) and benzoyl peroxide (10 %) were found to increase the epidermal thickness and to decrease the number epidermic comedones. Treatment with retinoic acid leads to a cleaning of the skin due to comedone expulsion and to a modification of the dermic cyst population. With benzoyl peroxide, the dermis showed a thickening, probably due to oedema formation and as a consequence the pseudocomedones were squeezed into elongated column like shapes. This simple and accurate assay can be recommended for the evaluation of other comedolytic agents used in the treatment of Acne.

Anticarcinogenesis: Possibilities for modulation of initiation by modulation of metabolism.

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The chemical species immediately responsible for initiation is usually controlled by activating, inactivating and precursor sequestering enzymes. One of the structural elements which are widely occurring in very many chemical mutagens and carcinogens are aromatic and olefinic moieties. These can be transformed into epoxides by microsomal monooxygenases. Such epoxides may spontaneously react with nucleophilic centers in the cell and thereby covalently bind to DNA, RNA and protein. Such a reaction may lead to cytotoxicity, allergy, mutagenicity and/or carcinogenicity, depending on the properties of the epoxide in question. An important contributing factor is the presence of enzymes controlling the concentration of such epoxides. There are several microsomal monooxygenases which differ in activity and substrate specificity. With large substrates, some monooxygenases preferentially attack at one specific site different from that attacked by others. Some of these pathways lead to reactive products, others are detoxification pathways. Also important are the enzymes which metabolize epoxides, such as epoxide hydrolases and glutathione transferases. Such enzymes can act as inactivating and in some specific cases also as co-activating enzymes. Moreover, precursor-sequestring enzymes such as dihydrodiol dehydrogenase, glucuronosyl transferases and sulphotransferases are important for the control of reactive epoxides. These enzymes themselves are subject to control by many endogenous and exogenous factors. By virtue of their contribution to the control of carcinogenic metabolites such modulators can be used experimentally to investigate the role and relative importance of various carcinogen-metabolizing enzymes and can also act as modifiers of initiation.

ANTICARCINOGENS AS AN APPROACH FOR SKIN CANCER PREVENTION.

Hasan Mukhtar. Departments of Dermatology, Case Western Reserve University and Veterans Administration Medical Center, Cleveland, OH, USA.

There is increasing interest in identifying naturally occurring as well as synthetic chemical agents that may be capable of substantially diminishing the tumorigenic potency of selected known carcinogens. Such chemical agents are known as anticarcinogens. Polycyclic aromatic hydrocarbons (PAHs), of which benzo(a)pyrene (BP) is a prototype, are ubiquitous environmental pollutants and are known skin carcinogens. PAHs are not carcinogenic themselves but require metabolic activation of the parent compound to highly reactive diol-epoxides, the DNA binding of which correlates with tumor risk. Metabolic biotransformation of PAHs is carried out by a series of enzyme reactions catalyzed by cytochrome P-450-dependent aryl hydrocarbon hydroxylase (AHH) and by epoxide hydrolase. Knowledge of the importance of these pathways in the initiation of PAH carcinogenesis has led to the suggestion that nontoxic inhibitors of AHH activity and/or subsequent macromolecular binding of the ultimate carcinogenic metabolites to DNA may reduce PAH cancer risk. We have shown that a series of naturally occurring plant phenols including ellagic acid, tannic acid, myricetin, quercetin, anthraflavic acid, green tea polyphenols, silybin and nordihydroguaiaretic acid and synthetic imidazoles such as clotrimazole and ketoconazole are potent inhibitors of the metabolism and/or DNA-binding of model PAHs. Pretreatment of the skin with selected topically applied agents affords varying degrees of protection against BP, benzo(a)pyrene 7,8-diol (a precursor of the ultimate carcinogenic bay region diol epoxide metabolite of BP) and DMBA-induced skin tumorigenicity assessed in a standard initiation-promotion protocol and against 3-methylcholanthrene-induced skin tumorigenicity assessed in a complete carcinogenesis protocol. Tannic acid has been shown to possess exceptional activity in this regard. These observations suggest the exciting possibility that the addition of anticarcinogenic substances to cosmetic products, soaps and medications may diminish the risk of environmentally induced human skin cancer.

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## EXPERIMENTAL MULTISTAGE CARCINOGENESIS: THE BASIS FOR THE DEVELOPMENT OF NEW CONCEPTS IN CANCER CHEMOPREVENTION

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The multistage approach of experimental carcinogenesis in mouse skin - consisting of the stages initiation, conversion, promotion and malignant progression - is characterized by the subsequent development of epidermal hyperplasia, non-autonomous and autonomous papillomas, and carcinomas. Progression to the malignant state is a late and low-yield event, which depends on the pre-existence of papillomas. Papilloma development is induced by chronic hyperplasiogenic stimulation (promotion) of skin which has previously undergone initiation and conversion. Conversion is achieved by a single treatment of skin with certain agents such as the phorbol ester TPA (or by wounding) before or after initiation. The converted state of skin, as characterized by increased promotability, is slowly reversible. If promotion is inhibited or interrupted before autonomous papillomas appear, the progress of skin tumorigenesis exhibits a high degree of remission. A prevention of conversion or promotion, or an interruption of promotion thus bring cancer development to a halt, although the initiated state is virtually irreversible.

Conversion and promotion are inhibited by inhibitors of the arachidonic acid cascade (cyclooxygenase as well as lipoxygenase pathway), retinoids and corticosteroids. Promotion can also be prevented by antioxidants and inhibitors of proteinkinase C. Especially powerful antipromoting agents are the cyclosporins, regardless of whether they exhibit immunosuppressive efficiency or not.

## ANTICARCINOGENESIS: PHOTOPROTECTIVE ROLE OF MELANIN. M.A. Pathak, Department of Dermatology, Harvard Medical School, Boston, MA, USA.

There are a number of well documented clinical and experimental observations that indicate the chronic effects of exposure to UVR causes nonmelanoma and melanoma skin cancer in fair-skinned individuals of Skin Types I - III who burn easily and tan poorly or minimally. The remarkable resistance of pigmented skin (Type IV - VI) to the carcinogenic effects of UVR is related to melanin. The type of melanin (eumelanin and not pheomelanin) and its amount and distribution in the human epidermis is the single most important factor in the protection of human skin against UVR carcinogenesis. A number of plausible mechanisms have been proposed to explain its protective effect. Melanin is not an anticarcinogenic or anti-promoting agent; it is a protective biopolymer distributed in the basal and suprabasal layer of epidermis in a particulate form as melanosomes and as colloidal macromolecular form dispersed in the malpighian cells and stratum corneum to act as: (1) a filter that absorbs harmful radiation and attenuates the impinging radiation by absorption, scattering, and subsequent dissipation of the absorbed energy as heat; (2) an absorber of UVR and acting by a redox system in the cellular oxidation-reduction reaction; (3) as a stable free radical that regulates the electron-transfer processes; and (4) as a pseudo dismutase that acts as a scavenger of superoxide anion generated by UVR. In living cells of human skin, UVR causes damage to DNA and generates reactive $O_2$ and free radicals ($^1O_2$, $O_2^-$, $^{\cdot}OH$, and $H_2O_2$) that damage cell membranes, essential enzymes, and chromosomes. Melanin by means of its capacity to undergo immediate oxidation-reduction reactions protects that melanized epidermis against the reducing and oxidizing conditions resulting from the generation of reactive $O_2$ species and free radicals. One advantage of UV exposure is the induced response (thickened stratum corneum and increased melanin) that confers protection against the effects of subsequent exposures.

## EXTEMPORANEOUS AND PROPRIETARY DILUTION OF CORTICOSTEROID OINTMENTS - PRACTICAL CONSIDERATIONS CONCERNING POTENCY.

J. R. Gibson, J. E. Hough, P. Marks and A. Webster, Skin Department, The London Hospital and Wellcome Research Laboratories, Kent, England.

It has been demonstrated recently that the human vasoconstrictor assay, with suitable refinements, may be used to predict with reasonable accuracy the relative clinical potencies of corticosteroid preparations in the treatment of human disease and that its utility goes well beyond that of a crude model for the selection of potent corticosteroids for clinical use. The aim of this work was to define dose response curves for serial dilutions of two commonly used corticosteroid ointments in order to provide general guidance concerning the degree of dilution needed in order to achieve changes of practical significance in corticosteroid ointment potency.

Readings of the human vasoconstrictor assay using three independent scorers were obtained at intervals over a 24-hour period for serial dilutions of clobetasol propionate 0.05% (Dermovate) and betamethasone valerate 0.1% (Betnovate) ointments in their recommended diluents in two separate studies.

The dose response curve generated for the clobetasol propionate ointment series indicated clearly that there is a threshold point beyond which significant concentration increases produce relatively small changes in potency. The results from the betamethasone valerate ointment series yielded similar data.

Our conclusions are that it is unlikely that dilutions of one part in three of many marketed ointment formulations will yield changes of practical significance and, as a "rule of thumb", dilutions of at least one part in four are needed for clinically detectable changes to occur. Clinicians should be aware that some currently used proprietary and extemporaneous dilutions of corticosteroid ointments are likely to have virtually the same clinical potency and adverse effect profile as the original preparation.

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## SKIN PERMEABILITY USING HYDROCORTISONE LOTION IN TWO DERMATOSIS MODELS.

To study skin permeability, two experimental dermatoses were induced in hairless rats : that produced by cellotape stripping and that invoked by dietary essential fatty acid deficiency (EFAD) .
A hydro-alcoholic lotion of 1 % hydrocortisone (HC) labelled with C-14 was applied to a 4.5 $cm^2$ area on the back under occlusion . Animals were sacrificed at different periods and serum concentrations of HC and its metabolites were determined.
Two pharmacokinetic parameters were calculated :the maximal concentration ($C_{max}$) and the time to obtain this maximal value ($T_{max}$) .
In stripped rats, the $C_{max}$ (15 X $10^{-7}$ M) was about 100 fold higher than the value observed in control rats . A large difference in $T_{max}$ values was also noted between the stripped rats ($T_{max}$ = 20 minutes) and the controls ($T_{max}$ = 4 hours) .
In the EFAD rats, the $C_{max}$ (3 X $10^{-7}$ M after 77 days on deficient diet and 1 X $10^{-7}$ M after 22 days) was about 20 and 8 fold higher respectively, than the mean control value (1.3 X $10^{-8}$ M) . In this model, however the $T_{max}$ (about 3 hours) was quite similar to the control value (4 hours) .
Our results underline that the horny layer barrier is primarily responsable for regulating the penetration of HC . When this barrier is removed, molecules penetrate and are resorbed quickly and massively . When this function is only disturbed as in EFAD rats, $C_{max}$ increases relative to the degree of deficiency whereas $T_{max}$ remains nearly unaffected .

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INHIBITION OF NEUTROPHIL CHEMOTACTIC FACTOR RELEASE FROM GUINEA PIG SKIN BY DEXAMETHASONE IN ANAPHYLACTIC REACTION.

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A prolonged inflammatory reaction after immediate wheal and flare in anaphylaxis in skin might be due to inflammatory cell infiltration in the site of the reactions. In the previous study, we demonstrated that neutrophil chemotactic factor (NCF), as well as histamine, is released from guinea pig skin in anaphylactic reactions, and it is leukotriene $B_4$-like substance. The prolonged inflammatory reactions in the cutaneous anaphylaxis is generally suppressed by corticosteroids.

In the present study, in order to analyze the mechanism of the action of corticosteroids in the prolonged inflammatory reactions in the cutaneous anaphylaxis, the effect of dexamethasone (DM) was investigated on the in vitro anaphylactic release of NCF from guinea pig skin.

Skin slices obtained from guinea pigs actively sensitized with egg albumin was preincubated in RPMI 1640 containing $10^{-5}$M DM and 10% heat inactivated guinea pig serum for 12 hours at 37 °C in 5% $CO_2$-air atmosphere. The preincubated slices were washed in calcium-free Tyrode solution and incubated in Tyrode solution containing egg albumin (50µg/ml) for 20 min at 37 °C.

The antigen-evoked NCF release was almost completely inhibited by preincubating the slices with $10^{-5}$M DM (mean inhibition: 88.6% ± 9.2 S.D.). In addition, the antigen-evoked NCF release was also inhibited by preincubating the slices for 40 min at 37 °C in the supernatant which was obtained by incubating the slices with DM and dialyzed (mean inhibition: 66.6% ± 25.3 S.D.).

These result suggest that the anaphylactic release of the NCF from guinea pig skin may be inhibited by the inhibitory factor produced by corticosteroids in the skin.

TRANSCUTANEOUS COLLECTION OF SYSTEMICALLY ADMINISTERED THEOPHYLLINE IN RHESUS MONKEYS.

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Transcutaneous chemical collection (TC) is a novel method, developed in our laboratory, for the non-invasive collection and measurement of body exposure to drugs and other chemicals. Our Transcutaneous Collection Device (TCD) is a circular adhesive-tape-encased saline-aquagel (per gram aquagel: 0.03 gm agarose, 0.05 gm of activated carbon, 0.92 gm 10% saline). In order to investigate the influence of skin site and skin contact time on TC, TCDs were emplaced on the closely clipped chests and abdomens of female Rhesus monkeys (RM: 2 monkeys for total of 5 studies, 14 days between studies on same monkey) prior to systemic aminophylline (A) administration (10 mg/kg of theophylline) by IV infusion over 30 min. The gel surface area in contact with skin was 1.33 $cm^2$. TCDs were placed 24 hr (n=10 per monkey) and 1 hr (n=10 per monkey) prior to the administration of A. Blood samples were collected at 0, 0.5, 2, 4, and 24 hr post-dose. TCDs were removed at 24 hr post-dose and assayed by HPLC for theophylline (T). The mean±SD of T per TCD for the 1 and 24 hr groups were 167±157 and 371±287 ng respectively. The average within monkey CVs for the 1 and 24 hr groups of patches were 87 and 57%. ANOVA was used to assess the difference in accumulated collection of theophylline (T) due to pre-dose emplacement time and skin site location of TCD placement. TCDs placed 24 hr prior to dosing had significantly higher T accumulation than TCDs placed at 1 hr before dosing ($p<0.005$). No significant differences were found which were attributable to skin site. *In vivo* permeability coefficients (Kp x $10^4$ cm/hr) were 5±2.9 and 11±5 for 1 and 24 hr groups respectively ($p<0.005$). We conclude: (1) that there is no skin site effect of TCDs located on the chest or abdomen of RMs and (2) emplacement of TCDs 24 hr prior to T administration yields greater skin permeability & flux, probably due to hydration of stratum corneum.

DRUG-INDUCED EXPERIMENTAL PORPHYRIAS: ANIMAL MODELS FOR HUMAN HEPATIC PORPHYRINS.

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Drugs can induce in experimental animals two different types of hepatic porphyria which closely resemble two human porphyrias with cutaneous manifestations.

Griseofulvin and a substituted dihydropyridine (DDC) both cause marked inhibition of liver ferrochelatase and accumulation of protoporphyrin in the liver of mice, and also in their circulating erythrocytes, thus providing a model for human Erythropoietic protoporphyria. In contrast, hexachlorobenzene and other polyhalogenated chemicals lead to inhibition of hepatic uroporphyrinogen decarboxylase and uroporphyrin is the main metabolite to be excreted: this second type of experimental pophyria is a good model for human porphyria cutanea tarda.

The mechanism by which griseofulvin and DDC induce hepatic porphyria has recently been clarified: these drugs convert the haem of hepatic cytochrome P-450 into an alkylated porphyrin, N-methyl protoporphyrin, a selective and powerful inhibitor of ferrochelatase. The mechanism of action of hexachlorobenzene is, on the other hand, still obscure, but recent work has again emphasized the role of a non-haem iron pool in the pathogenesis of the condition and suggested the importance of reactive oxygen species produced in the liver by the interaction of the drug with a cytochrome P-450.

TOXICITY OF LASER-ACTIVATED HEMATOPORPHYRIN DERIVATIVES TO CULTURED HUMAN EPIDERMAL KERATINOCYTES

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Cultured keratinocytes are a good tool to study drug toxicity. We applied this cellular model to study the effects of hematoporphyrin derivatives (HPD) which are used as photosensitizers in tumor therapy. Keratinocytes were isolated from human skin and cultured for several weeks. They were incubated for 60 min with 5-20 µg/ml HPD. After centrifugation the cells were washed and suspended in cell culture medium. All steps were carried out in the darkness. Cells were irradiated with an He-Ne-laser (6 mW) at 632 nm and 0 °C, applying up to 5 Joule. Afterwards cell viability, enzyme release and lipid peroxidation were followed for 1 h. Cell viability (85-90 % keratinocytes excluded trypan blue) did not change alone with HPD or laser light respectively. But after treatment with both up to 70 % were damaged as indicated by trypan blue uptake and lactate dehydrogenase release. Both parameters depended on the concentration of HPD and the intensity of laser light. But no malondialdehyde, a parameter of lipid peroxidation, was formed, although control experiments with HPD and laser showed a concentration- and light intensity-dependent formation of hydroxyl radicals as indicated by the release of ethene from methional. On the other hand, acid phosphatase activity, a lysosomal enzyme, increased considerably when measured in the supernatant of the cell suspension, indicating damage of lysosomes. The results suggest that HPD in the presence of laser light do not initiate lipid peroxidation in keratinocytes. Therefore, cytotoxicity must be related to other mechanisms, e.g. direct lysosomal damage resulting in the release of a number of enzymes which degrade cellular constituents. However, other cellular targets, e.g. nuclear DNA, cannot be ruled out.

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## CHEMICAL CARCINOGENS AS CONTACT SENSITIZERS IN MICE.

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Induction of skin tumors in mice by the application of polyaromatic hydrocarbons to the skin has been widely used to study mechanisms of chemical carcinogenesis. Most stategies examining the role of the immune system in this process have focused on immune responses of mice to the resultant carcinogen-induced tumors. The purpose of this study was to examine the cell-mediated immune response of adult C3H mice to two carcinogenic PAHs, dimethylbenzanthracene (DMBA) and benzo(a)-pyrene B(a)P, in order to provide a basis for examining the participation of the immune system in the initial stages of chemical carcinogenesis. Allergic contact sensitization could be produced by epicutaneous application of either DMBA or BaP under occlusion to the shaver abdomens of naive mice. Ear challenge of DMBA-sensitized mice with DMBA produced a significant ear swelling response when compared to nonsensitized controls ($11.6 \pm 1.5 \times 10^{-3}$ vs. $-0.5 \pm 0.6 \times 10^{-3}$cm). BaP challenge of BaP-sensitized and non-sensitized mice produced a comparable pattern ($9.4 \pm 1.5 \times 10^{-3}$vs. $-1.9 \pm 0.2 \times 10^{-3}$cm). Histologic sections of challenged ears from sensitized mice revealed an extensive mononuclear infiltrate consistent with a cell-mediated immune response. Dose-response evaluations using DMBA revealed that optimum sensitization occurred using 100 ug DMBA. Both higher and lower sensitizing doses elicited lesser responses. A similar pattern was observed for BaP. This system in which PAHs are both carcinogenic and contact sensitizers provides an excellent model in which to evaluate the interaction of cell-mediated immune responses to chemical carcinogens during the initial stages of tumor induction by the same chemicals.

## CURRENT DEVELOPMENTS IN DIAGNOSTIC OF DRUG ALLERGIES AND PSEUDO-ALLERGIES

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In vitro tests for the diagnosis of drug allergies and pseudo-allergies have remained and still are a difficult and unsatisfactory area of clinical allergy. Among the reasons responsible for this state of affairs are: the problem of identifying the complete drug-protein carrier allergens formed in vivo, the genetic conditioning of allergic responses to drugs, the questionable role of IgE and IgG antibodies to drugs, the difficulties in detecting cellular immune responses to drugs.

In recent years, various serological techniques have been used and compared for the serologic detection of IgE and IgG drugspecific antibodies to β-lactam antibiotics, sulfamides, pyrazolone derivatives and myorelaxants, among others. These results will be briefly reviewed and discussed. For assessment of cellular responses to drugs, basophil degranulation tests, lymphocyte transformation tests and the determination of some lymphokines, such as the lgukocyte migration inhibition factors (LIF) have been mostly in the forefront. The development of additional tests such as histamine release, the procoagulant test, lymphocyte and platelet activation test, as well as newer techniques enabling to detect cellular activation at the single cell level will be briefly presented.

## CYTOCHROME P-450 ISOZYMES : TARGET FOR AZOLE DERIVATIVES.

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An amazing number of N-substituted imidazole and triazole derivatives are antifungals of use in plant protection, veterinary and human medicine. Their antifungal activity might originate from interactions, at nanomolar concentrations, with a cytochrome P-450 (P-450) involved in the 14-demethylation (C-32 demethylation) of lanosterol or 24-methylene dihydrolanosterol. This is a key step in the biosynthesis of ergosterol, the main sterol in yeast, fungi and some protozoa, e.g. Leishmania. At higher concentrations than those required for antifungal therapy, the azole antifungals also interfere with the C-32 demethylation of lanosterol in mammalian cells. Some of the imidazole derivatives (e.g. ketoconazole), but not the triazole derivatives (e.g. itraconazole) investigated so far, interact at high concentrations with some P-450 isozymes and P-450-dependent pathways in testis, adrenals, liver, kidney and skin. The effects of high doses of ketoconazole (ketoconazole HD) on the testicular P-450-dependent 17,20-lyase are of special interest. Indeed ketoconazole HD inhibits androgen biosynthesis. This property makes ketoconazole HD a suitable candidate for the treatment of some androgen-dependent diseases. This finding was certainly at the onset of an intensive search for selective affectors of P-450 isozymes in microorganisms, plants and mammals. The P-450 studies already helped in the development of a selective orally and topically active antifungal, itraconazole. It is hoped that this research will result in new possibilities in medical treatment.

## PROTECTION AGAINST CHEMICALLY INDUCED SKIN TUMORIGENESIS BY NATURALLY OCCURRING PLANT PHENOLS IN SENCAR MICE.

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Polyaromatic hydrocarbons (PAHs) including benzo(a)pyrene (BP) require metabolic activation to reactive intermediates which bind covalently to DNA in order to exert their tumorigenic effects. Our prior studies have shown that a series of naturally occurring plant phenols inhibit the metabolic activation of PAHs and their binding to epidermal DNA. In this study these plant phenols including tannic acid (TA), quercetin (QT), myricetin (MY), anthraflavic acid (AA) and ellagic acid (EA) were tested for their skin antitumorigenic activity in SENCAR mice. TA, QT, MY, AA and EA resulted in significant protection against dimethylbenz(a)anthracene (DMBA) and BP induced skin tumorigenesis. A significant increase in the latent period for the development of skin tumors was observed in the plant phenols treated groups as compared to the control group of animals receiving DMBA or BP alone. After 12 weeks of testing, the number of tumors/mouse in the groups receiving plant phenols were significantly lower than the corresponding controls receiving DMBA or BP alone. We further argued that if the protective effects of plant phenols on PAHs induced skin tumorigenicity is entirely related to the inhibition in the metabolism then these plant phenols should be devoid of their inhibitory effect on skin tumorigenesis induced by methyl nitrosourea (MNU), which do not require metabolism to elicit its tumorigenic effect. However, this was not the case as all the plant phenols tested showed significant protection against MNU induced skin tumorigenicity. These results suggest that these plant phenols have antitumorigenic effect against variety of carcinogens and one possible mechanism may be due to inhibition in the alteration of target tissue macromolecules.

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EFFECT OF CIMETIDINE ON MURINE ALLERGIC CONTACT HYPERSENSITIVITY (ACH)

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Treatment with cimetidine during the induction but not the elicitation of ACH produces a marked enhancement and prolongation of the response. This observation was further evaluated in BALB/c mice treated with cimetidine (100 mg/kg) or saline, intraperitoneally, twice daily from days 0 to 3 and sensitized with 0.1% trinitrochlorobenzene (TNCB) on day 0 to the nape of the neck. On day 7, the mice were challenged with 1% TNCB to one ear. Ear swelling measurements in groups of 4 to 6 mice were obtained at 0, 0.5, 1, 1.5, 2, 4 6, 8, 12, 24, and 48 hrs after challenge. At the same time points, serum histamine was measured by a radioenzyme assay, and frozen and paraffin sections of ears were stained with H&E or Giemsa reagent to assess the degree of granulation of mast cells and nature of the cellular infiltrate. Control mice included animals challenged with but not sensitized to TNCB. Cimetidine-treated mice had increased ear swelling at all time points as compared to saline controls: $10.1 \pm 1.1 \times 10^{-2}$ mm, $13.2 \pm 1.0$, and $19.8 \pm 1.6$ (mean $\pm$ SEM) for saline-treated mice vs $15.1 \pm 0.8$, $18.5 \pm 0.6$, and $29.8 \pm 1.2$ for cimetidine-treated mice at 1.5, 8, and 24 hrs respectively. No significant difference in serum histamine was noted between the cimetidine-and saline-injected mice at any time; both groups displayed a biphasic histamine response with peaks between 0.5 and 4 hrs and a second peak between 8 and 12 hrs, as we have previously described. Histologically, the cimetidine-treated animals showed a more intense cellular infiltrate than the saline controls which was most noticeable at 24 hours and characterized by numerous subcorneal and perifollicular neutrophilic abscesses. There was no difference in mast cell morphology between the two groups. We conclude that enhancement of ACH by cimetidine is independent of an effect on mast cells, but may relate to a cimetidine-induced inhibition of T-suppressor cells at the time of sensitization.

THE INFLUENCE OF $Ca^{++}$ MODULATORS ON EPIDERMAL CELLS IN VITRO. A protective effect from Ca overload?

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Both, proliferation and differentiation, are significantly influenced by $Ca^{++}$ ions in vitro. Modulators of cellular $Ca^{++}$ homeostasis, e.g. $Ca^{++}$ antagonists, are potent drugs to prevent heart and smooth muscle cells as well as fibroblasts from damage and necrosis by excessive $Ca^{++}$ influx. Recently, a new class of $Ca^{++}$ modulators has been described - $Ca^{++}$ agonists - which possess pharmacological effects diametrically opposed to those of $Ca^{++}$ antagonists, To our knowledge, there is no information about the effects of these drugs on epidermal cells.

In the present study cultured guinea pig epidermal cells were seeded on collagen-coated dishes and maintained under low (1.0 mM) and high (2.7, 3.6 mM) $Ca^{++}$ conditions. The cells were continuously exposed to the $Ca^{++}$ antagonists nifedipine (Adalat(R), 0.2 0.5, 1.0 µg/ml) and verapamil (Isoptin(R) 0.2, 0.5, 1.0 µg/ml), and to the $Ca^{++}$ agonist BAYK 8644 (0.2, 0.5, 1.0 µg/ml). Cell growth was monitored by cell counting and $^3$H-thymidine labeling and related to the cell-bound $Ca^{++}$ measured by flame photometry of homogenized cells.

Nifedipine and BAYK 8644 dose-dependently reduced DNA-synthesis and the cell number under low $Ca^{++}$ conditions, whereas verapamil was uneffective compared to untreated controls. The cell-bound $Ca^{++}$ was reduced by nifedipine (50 %), but not by verapamil and BAYK 8644. In comparison to low $Ca^{++}$ cultures, high $Ca^{++}$ cultures showed a lower plating efficiency, less cell spreading, distinct cytoplasmic vacuolization, an increased number of trypan blue stained cells to 12 %, and reduced cell growth. Nifedipine was able to counteract all these changes caused by high $Ca^{++}$ medium, whereas verapamil and BAYK 8644 did not.

The results let us suggest that of all tested drugs only nifedipine had an effect on epidermal cells mediated by $Ca^{++}$ metabolism. Its use may be beneficial to protect epidermal cells from cell death by $Ca^{++}$ overload.

CYCLOSPORINE IN PSORIASIS: NOVEL INDICATIONS OF KNOWN DRUGS.

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Oral cyclosporine A (CsA), 14 mg/kg/day or its vehicle were randomly assigned in a double blind fashion to 21 patients with severe psoriasis vulgaris. After four weeks of therapy, of eleven CSA recipients, two totally cleared, six improved markedly, two improved moderately and one improved minimally; whereas ten vehicle-treated patients showed no change or minimal improvement (P=0.0003). Vehicle-treated patients, after a switch to CsA for four weeks, demonstrated impressive improvement similar to that seen in patients who initially received only CsA. Moderate or marked improvement or total clearing was noted in 17 of 21 patients (81%) and 20 of 21 (95%) after one and four weeks of therapy, respectively. Mitotic figures and leukotriene $B_4$ levels in lesions decreased 86% and 64%, respectively, after seven days of CsA therapy. These results suggest that: (1) psoriasis may have an immunologic basis and (2) if a long-term regimen with a favorable efficacy:side effect ratio can be determined, CsA would be a significant advance in the treatment of psoriasis.

IN VITRO AND IN VIVO ANTI-INFLAMMATORY ACTIVITIES OF C10 SUBSTITUTED ANTHRALIN DERIVATIVES.

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Anthralin (1, 8, dihydroxy-9-anthrone) has been a very effective drug in the treatment of psoriasis for more than 60 years. However, it suffers many drawbacks to its widespread use, including chemical instability and skin irritation. In recent years efforts have been made to overcome these problems by introducing pro-drug forms, however, these are either too slow in action (e.g. the tri-acetate) or too difficult to control in their formation of anthralin (e.g. butanthrone). We have recently compared three members of a family of C10 succinyl derivatives of anthralin, with anthralin in a variety of in vitro and in vivo test systems. In those test systems where anthralin activity was related to its potent anti-hyperproliferative activity (inhibition of ornithine decarboxylase induction in vivo and keratinocyte metabolism in vitro), these three analogues were inactive. However, in anti-inflammatory test systems, both in vitro and in vivo and particularly those related to the inhibition of the metabolism of arachidonic acid via both the cyclooxygenase and lipoxygenase pathways these compounds were active and comparable with reference standards. Furthermore these compounds show a highly significant improvement, relative to anthralin, with respect to local irritation and systemic toxicity and in addition do not result in staining of either skin or cloth.

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## THE EFFECTS OF ISOTRETINOIN ON FOLLICULAR AND SEBACEOUS GLAND DIFFERENTIATION.

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Isotretinoin is known to reduce the rate of sebum secretion and to shrink sebaceous glands. How these effects are caused and whether the response in acne also depends on an effect on follicular keratinisation is unknown.

Non-lesional skin from the acne bearing area of the back from 17 acne patients was examined at 4 or 8 weeks of isotretinoin treatment. Glucose-6-phosphate dehydrogenase (G6PDH), succinic dehyrogenase (SDH) and non specific esterase (NSE) reaction products were measured in interfollicular epidermis, follicular and sebaceous epithelium, as indices of metabolic activity. Protein synthesis was assessed by a quantitative autoradiologic method using tritiated leucine. The volumes of sebaceous gland, differentiated and undifferentiated sebaceous epithelium and follicular epithelium were measured by a stereological point counting technique. Sebaceous gland cell size and hair follicle orifice area were also measured.

There was a significant reduction in the reaction products of G6PDH and SDH activities in sebaceous glands but no change in interfollicular or follicular epithelium after treatment. No change in protein synthesis could be detected. There was a significant decrease in the volume of sebaceous glands and differentiated sebaceous epithelial cells. The ratio of differentiated to undifferentiated sebaceous cells fell significantly during treatment. There was no change in the volume of the hair follicle or follicular epithelium or in the hair follicle orifice area.

The results confirm that isotretinoin reduces sebaceous gland volume and demonstrate that the glands also have a reduced metabolic activity after isotretinoin treatment. No change in follicular epithelial differentiation was detected by the methods used. Etretinate has been shown to alter the metabolic activity in interfollicular epidermis, indicating one difference in the mode of action of these two retinoids.

## A NEW CHEMICALLY STABLE RADIO-LIGAND (CD270) AND ASSAY PROCEDURE FOR CYTOSOLIC RETINOIC ACID BINDING PROTEIN.

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The biological and therapeutic activity of retinoids is believed to be related to their ability to bind to a cytosolic protein receptor - cytosolic retinoic acid binding protein (cRABP), which participates in the nuclear translocation of the retinoids. Until now, [3H]-retinoic acid has been used to measure the binding and to quantitate the amount of cRABP in cell (or tissue) extracts. Retinoic acid (RA), however, is a very unstable molecule, sensitive to light and oxidation. This instability is increased by radiolysis, and in spite of the precautions taken, breakdown of RA is an ever-present problem.

We have developed a new retinoid that can successfully substitute for retinoic acid as a ligand for cRABP. The new retinoid 2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphtyl)-6-benzo-[b]-thiophenecarboxylic acid, coded CD270, is labelled specifically with tritium on the naphthalene moiety and has a specific activity of 7.6 Ci/mmol. This retinoid is chemically stable and insensitive to light or atmospheric oxidation.

At the same time, a new assay procedure has been developed to measure the binding to cRABP. The method allows a complete separation between the bound and free retinoid by means of gel filtration on small columns of Sephadex G-25. A single fraction (of the cRABP-retinoic complex) is collected from each column into a scintillation counting vial. A single person can run 60-100 samples per day (4-6 binding curves). This assay is used to measure the binding in either saturation- or competition binding experiments. CD270 binds to cRABP from several sources (human, rat, mouse, bovine) with affinity similar to that of RA ($K_d$ of about 2 nM for both). When measuring the affinities of unlabelled retinoids by competition binding, the results obtained were the same irrespective of whether RA or CD270 was used as the radioactive ligand. When cRABP was quantified in human epidermal keratinocytes and in cytosol from rat and bovine testes, again the results obtained with CD270 were similar to these obtained with RA.

These experiments confirm that CD270 binds to cRABP with an affinity and specificity similar to these of RA. We propose, therefore, [3H]-CD270 as a chemically stable ligand for cRABP, that can successfully substitute for RA in most binding studies.

## A NEW METHOD TO STUDY PRURITUS AND ANTIPRURITIC DRUGS.

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A major obstacle in the study of clinical itch and its therapeutic control, is the lack of accurate methods for its measurement. In this study we have used a new method based on a micro computer (Pain-Track®) in an attempt to increase reliability and compliance of subjective ratings of pruritus. Pain-Track is a portable data logger (8.9x14x2.9 cm; 300 g). With a standard battery of 9.5 V, a continuous recording for at least 8 weeks can be made. Every 60 min a buzzer commands the patient to mark his presence on a marker button and rate the itch intensity on a knob with a fixed point scale from 0 to 6 (0=no and 6=maximal itch). During nighttime the hourly buzzer can be turned off, but the intensity rate knob can still be changed whenever wanted. When the recordings are finished the data logger is plugged into a desk-top computer for storage and analysis of the collected information.

In a double-blind, cross-over study 30 adult out-patients with persistent atopic dermatitis were treated with betamethason dipropionate or its corresponding cream base. We measured the itch intensity during each period (4 days) of treatment, both continuously by Pain-Track and retrospectively each day by conventional diary cards. The overall compliance with Pain-Track was 90%. In 19 of the 20 pts who had a compliance rate of >80% the itch intensity measured by Pain-Track was less during active treatment than during placebo. In many cases the decreased itch was recorded already during the first day of treatment. Moreover, there was a good correlation between the clinical picture and itch intensity.

Conclusion: By using a drug with known antipruritic effect in atopic dermatitis we have shown that Pain-Track is a useful tool for assessing clinical pruritus and the antipruritic effects of drugs. The main advantages of the new method are possibilities for: 1) frequent recordings, 2) surveillance of compliance, 3) storage and analysis of a large amount of data.

## Calcium Channel Antagonists in Dermatology

Smooth muscle contraction is calcium dependent. Calcium influx into the smooth muscle cell can be blocked by a group of drugs which block the 'slow' calcium channels. Of these drugs nifedipine, verapamil and diltiazem have powerful effects on both the coronary and peripheral vasculature - hence their use in cutaneous disorders in the aetiology of which vascular or smooth muscle spasm are implicated.

Nifedipine has been most extensively used in Raynaud's phenomenon. It must now be regarded as a first line drug in the management of severe symptomatic Raynaud's phenomenon, providing symptomatic relief in approximately 60% of patients. In resistant patients its use in combination with prostacyclin analogues can result in symptomatic relief and improvement of cutaneous blood flow.

Severe chronic idiopathic perniosis has hitherto been resistant to therapy but recently nifedipine (modal dose 60 mg orally daily) has been demonstrated to relieve symptoms, promote healing and prevent occurrence of further lesions in approximately 70% of patients with previously unremitting disease.

The severe pain of leiomyomata has also been relieved by nifedipine in a small number of patients and its use in this disorder merits further investigation.

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Notices

**IMPROVEMENT AND VALIDATION OF IN-VITRO METHODS FOR ROUTINE TESTING IN DERMATO-TOXICOLOGY.**

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The use of animals in biological experiments as well as in dermato-toxicological investigations worldwide has become an issue of intensive public discussion. In contrast to an increasing number of papers on new alternative methods for RRR (Refinement, Reducement, Replacement) of animal experiments, there is a lack of in-vitro systems which have been validated sufficiently to justify their use in routine testing. Based upon recent work in our own laboratory and on previous papers by other authors three in-vitro systems of different complexity were selected for standardization and validation in a two year collaborative study, sponsored by the Federal Ministry for Research and Technology: a) cell culture, b) skin culture, c) chorion allantoic membrane (CAM) of incubated hen's eggs. All these systems proved to be suitable for routine testing. Cell and skin cultures provide good results when used for comparative testing within the same class of chemicals. But both methods have limitations with respect to chemical and physical properties of the products and chemicals to be tested. Best results were obtained with the chorioallantoic membrane. As this system seems most appropriate for further standardization with the aim to replace animal tests for skin and eye irritancy, further studies on improvement of evaluation methods and on defining a system of yardstick chemicals are performed presently. It is obvious, that no in-vitro system can be expected to meet all requirements of all different problems. But for a lot of aspects of testing for local effects there is a good chance to replace animal tests by CAM-testing in the near future.

**INTERFERONS IN DERMATOLOGY.** G. Mahrle, H.-J. Schulze, H. Rasokat, B. Thiele, H.F. Merk, Dept. of Dermatology, University of Cologne, West Germany.

Interferons (IFN) modulate proliferation, differentiation and HLA-DR expression. Clinical studies on the effect of interferons proved some benefit regarding the treatment of viral infections and skin tumors, such as melanoma and basal cell carcinoma.

We report on (1) the effect of rIFN-gamma on growth, differentiation, and AHH activity of cultured keratinocytes and (2) its influence on HLA-DR expression and DNA-synthesis in psoriatic epidermis. We present our results obtained by two clinical trials, (A) phase II/III study on the effect of rIFN-alpha in AIDS and AIDS-related Kaposi's sarcoma (n = 24) and (B) phase I/II study on the effect of rIFN-gamma in 28 patients with melanoma, arthropathic psoriasis, condylomata acuminata, epidermodysplasia verruciformis, bowenoid papulosis, Behcet's disease, and mycosis fungoides.

IFN inhibited cell growth of keratinocytes in vitro but not in vivo in psoriatic epidermis. IFN-gamma induced expression of HLA-DR in keratinocytes in vitro as well as in vivo. rIFN-alpha caused regression of Kaposi's sarcoma in some cases but did not influence immunodeficiency. rIFN-gamma was of some benefit in the treatment of genital warts, bowenoid papulosis, and Behcet's disease but there was no response of psoriasis.

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## CELL CULTURE MODELS IN SKIN PHARMACOLOGY AND TOXICOLOGY.

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Human cells in culture are being used with increasing frequency as in vitro models for studying toxicological and pharmacological mechanisms at the cellular level. Changes in cellular properties such as the adhesiveness of anchorage-dependent cells to their culture support, cell membrane permeability, respiratory and overall metabolic activity as well as the capacity of the cells to proliferate or differentiate have been monitored under the influence of a given drug by measuring, for example, cell detachment, release of intracellular material (ATP, enzymes, DNA), exclusion of trypan blue or uptake of vital dyes, alterations in gas exchange and heat production as well as thymidine incorporation or the expression of specific differentiation markers.

In the present review the usefulness of the cell culture approach will be discussed by comparing the dose response of transformed human keratinocytes in vitro to different antipsoriatic treatments (anthralin, PUVA, glucocorticoids, and retinoids) with data obtained under therapeutic conditions in vivo. The following cellular parameters are taken into consideration: cell detachment, thymidine incorporation, glutamine dissimilation and cornified envelope competence as measures, respectively, of cytotoxicity, proliferative activity, cellular respiration and the capacity of the cells to undergo terminal differentiation.

The results indicate that, at therapeutic concentrations, anthralin and PUVA exert their antipsoriatic action via their cytotoxic potential by either inhibiting cellular respiration or DNA replication, whereas retinoids and glucocorticoids do not act directly as cytotoxic drugs but rather modulate the equilibrium between epidermal proliferation and differentiation by as yet unknown mechanisms. Furthermore, our data support the view that cell culture systems can provide a useful tool for the screening of new chemical derivatives of certain drugs.

## USE OF AIR-EXPOSED KERATINOCYTE CULTURE FOR PHARMACOLOGICAL PURPOSES.

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Using a conventional (submerged) culture system keratinocytes undergo differentiation, although to a degree which is lower than that seen under the in vivo situation. When cultured at the air-liquid interface using dead de-epidermized dermis (DED) as a substrate, keratinocytes were found to express morphological features of differentiation similar to those seen in vivo. Therefore, the latter system offers an attractive model for studying the processes involved in the regulation of epidermal differentiation and the effects of drugs on it.

The analysis of the lipid composition of normal keratinocytes cultured on DED revealed a great similarity of lipid pattern with that seen under the in vivo conditions. In contrast to cells cultured in a submerged culture system, which contain relatively large amounts of phospholipids and low quantities of ceramides, cells cultured on DED contain low amounts of phospholipids and high amount of sterols and ceramides, especially of acylceramides. Under both culture conditions, however, linoleic acid was present in much smaller quantities as compared to the in vivo situation.

Administration of retinoic acid (RA) to both normal and malignant (squamous carcinoma cells (SCC)) keratinocytes induced marked changes in the morphology of cells when cultured on DED. In normal keratinocytes the administration of RA led to an increase of a number of cell layers accompanied by a loss of the granular layer. Furthermore, the horny layer was transformed to a parakeratotic layer and also keratinization of individual cells was observed. In contrast to normal keratinocytes the addition of RA markedly reduced the number of cell layers of all three SCC studied. The morphology of these cells, however, was not significantly altered. These results suggest that similarly to the in vivo situation RA exerts distinctively different effects on the proliferation and differentiation of normal keratinocytes as compared to SCC cells.

## COMPARATIVE TOXICITY OF ANTIMICROBIAL AGENTS ON TRANSFORMED HUMAN KERATINOCYTES.

F.M. Tatnall, I.M. Leigh and J.R. Gibson, Department of Dermatology, The London Hospital, London, E1 1BB, England.

Experimental data suggest that some antimicrobial agents may have an adverse effect on several aspects of the tissue repair process including retardation of wound epithelialization. The aim of this study was to investigate the comparative cytotoxic effects of a range of antiseptics and antibiotics using human keratinocytes transformed by Simian virus 40 (SVK14 cells).

SVK14 cells were grown to semi-confluence by adding $6x10^4$ cells per petri dish. After 48 hours the cells were exposed to serial dilutions of the therapeutic concentrations (in parentheses) of each of the following agents: hydrogen peroxide (3%), cetrimide (1%), sodium hypochlorite (0.5%), povidone iodine (4%), neomycin (1%), bacitracin (50 units/ml), polymyxin B sulphate (10,000 units/ml). The cells were exposed to the drug for 15 minutes and then washed and incubated in the culture medium (RPMI 1640 plus 10% fetal calf serum) for 24 hours. Dead cells were then washed off and the viable adherent cells were trypsinised and counted in a Coulter counter.

At therapeutic concentrations none of the antibiotics were found to be cytotoxic whereas all of the antiseptics produced 100% killing of SVK14 cells. Dilutions of therapeutic concentrations of the antiseptics ranging between 1000- and 20,000-fold (depending on the agent) were needed in order to achieve no-effect levels. Calculations based on 100% killing values for the antiseptics indicate that their order of toxicity from highest to lowest is sodium hypochlorite, cetrimide, povidone iodine and hydrogen peroxide.

We conclude that this cell line may be useful in studying the epithelial cytotoxicity of drugs in vitro and that care should be exercised in the selection of antimicrobial agents for use in wound management.

## INFLUENCE OF DERMAL FIBROBLASTS ON EPIDERMALIZATION

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We recently describe a method of epidermalization in vitro that permits to obtain a well differentiated human epidermis and to quantify this epidermalization (1). With this human living skin equivalent model composed as in vivo of a dermis and an epidermis, we could demonstrate that fibroblasts from psoriatic patients induce the hyperproliferation of normal keratinocytes (2). In this study, we investigated the influence of normal dermal fibroblasts on epidermalization.

Normal human dermal fibroblasts are combined with collagen, serum and culture medium. Almost immediately a gel forms which the fibroblasts begin to contract. Within a few days, the contraction is stabilized and a tissue of firm consistency is formed : the dermal equivalent(DE) (3). In this three dimensional collagen matrix, fibroblasts are in a state of differentiation similar to that observed in vivo (4). At different steps of the rearrangement of the collagen matrix by fibroblasts, cells are killed by an osmotic shock. So, substrats of different consistency can be made, in which fibroblasts can be alive or dead. Epidermalization is then initialized by sticking on these substrats either very thin 1 mm in diameter skin punch biopsies (Epidermis and superficial dermis) or 2 mm in diameter biopsies made in succion blister roof (Epidermis alone).

-The epidermalization is better on a collagen matrix that have been previously reorganized by the fibroblasts, than on a simple collagen gel.

-The presence of the fibroblasts of the biopsy promotes the epidermalization when no living fibroblasts are in the DE, but this effect is masked when fibroblasts are alive in the DE.

-For a same collagen texture, the epidermalization is promoted when the fibroblasts of the dermal equivalent are alive.

Thus fibroblasts influence the epidermalization: they not only remodel the extracellular matrix, but also secrete growth factors. These results underline the importance of the fibroblasts in the dermal-epidermal interactions and the living skin equivalent provide to study these interactions. Because this human skin equivalent can be made simple or complex, the effects of pharmacological agents on epidermalization can be evaluated in this culture model either by their actions on keratinocytes itselves or through the treatment of the dermal fibroblasts.

(1) Coulomb et al. Br. J. of Dermatol., 114, 91-101, (1986). (2) Sasag et al. Science, 230: 669-672, (1985). (3) Bell et al. Proc. Natl. Acad. Sci. USA, 76 : 1274-1278, (1979). (4) Coulomb et al. Br.J. of Dermatol., 111, suppl. 27, 83-87, (1984).

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1,25 DIHYDROXYVITAMIN $D_3$ METABOLISM AND RECEPTOR CONTENT IN CULTURED HUMAN EPIDERMAL KERATINOCYTES AS A FUNCTION OF DIFFERENTIATION. S. Pillai, D.D. Bikle, P.M. Elias. Dermatology & Medicine Services, Veterans Administration Medical Center, San Francisco, California, USA.

Human foreskin keratinocytes produce 1,25 dihydroxyvitamin D ($1,25(OH)_2D$) in a hormonally-regulated fashion and contain receptors for $1,25(OH)_2D$. Here, we investigated how $1,25(OH)D_3$ production and $1,25(OH)_2D$ receptor content is linked to epidermal differentiation using cultured neonatal foreskin keratinocytes. At different stages of confluence, cells were serum-deprived for 24 hrs, and examined for production of $1,25(OH)_2D$ or $24,25(OH)_2D$ from $^3H$-25(OH)D, receptor content and affinity, and differentiation markers (transglutaminase (TG), involucrin (I), and cornified envelope (CE)). Whereby maximal production of $1,25(OH)_2D$ occurred after the cultures reached confluence, the highest specific activity of 1-α-hydroxylase occurred just before confluence, when the cells also began to express TG, I, and CE activities. In post-confluent, fully-differentiated cultures $1,25-(OH)_2D$ production declined dramatically along with TG and I as $24,25(OH)_2D$ production increased. Keratinocyte cytosols, prepared by high-speed centrifugation from pre-confluent, confluent and post-confluent keratinocytes, and examined for $1,25(OH)_2D$ receptors by equilibrium binding and sucrose gradient centrifugation, possessed receptors of the same sedimentation characteristics as chick intestinal cytosol preparations. Scatchard analysis displayed a single class of binding sites at all stages of growth, with pre-confluent cells displaying the highest receptor concentration (35 fmol/mg protein) of high affinity (kd-270 pM), confluent cells showing less (25 fmol/mg/-protein) of high-affinity receptors, and post-confluent cells displaying lower numbers (25 fmol/mg protein) of low-affinity receptors (kd-1400 pM). The correlation of $1,25-(OH)_2D$ production and receptor content with keratinocyte differentiation markers supports a regulatory role for vitamin D metabolites in epidermal differentiation.

A COMPARATIVE STUDY OF THE EFFECTS OF NSAID's, CORTICOSTEROIDS, RETINOIDS AND ANTHRALIN ON HUMAN PMN MIGRATION, OXIDATIVE BURST, DEGRANULATION AND 5-LIPOXYGENASE ACTIVITY.
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The acute inflammatory component of various skin disorders, e.g. psoriasis, is characterized by a pronounced invasion of the skin with PMN. Using human neutrophils, the influence of drugs with potential interest to dermatology was studied in vitro, in order to delineate their action spectrum on cellular activities of possible relevance to cutaneous inflammation. PMN migration (random and directed), lysosomal enzyme release and active oxygen production as well as 5-lipoxygenase activity. The cells were continuously incubated with the drugs when migration (chemokinesis and chemotaxis) stimulated by the chemotactic peptide f-MLP was studied, whereas a short (15 min) treatment of the cells preceded the assessment of cellular responsiveness to FMLP (oxidative burst, degranulation) or A23187 (5-lipoxygenase). Cellular movement was depressed by micromolar concentrations of most drugs including steroids, the strongest inhibitors being Indomethacin, Phenylbutazone, BW755C, Retinoic acid, Arotinoid and Anthralin. In contrast, 5-lipoxygenase activity remained unaffected after treatment by virtually all agents except known lipoxygenase inhibitors (Caffeic acid, NDGA, BW755C, ETYA, Benoxaprofen), and was marginally affected by large concentrations of Retinoic acid and Arotinoid. The oxidative burst, as measured by chemiluminescence in the presence of Luminol, and degranulation, as quantified by the release of lysozyme, were inhibited by some but not all cyclooxygenase inhibitors (e.g. Indomethacin, Phenylbutazone). Lipoxygenase inhibitors and retinoids (Arotinoid) were potent antagonists of active oxygen production. In the cases of Caffeic acid and BW755C, these effects could at least partially be explained by antioxidant properties of the molecules since similar observations could be obtained in a cell-free $O_2^-$-producing system. Degranulation was usually less sensitive to these drugs, retinoids being totally ineffective. Anthralin consistently gave bimodal dose-response curves in the chemiluminescence assay (mild stimulation at $10^{-6}$M, partial inhibition at $10^{-5}$M), and a slight stimulation of degranulation. Under these experimental conditions (short duration incubations), corticosteroids were almost devoid of any inhibitory activity. Taken together these results show that depression of PMN migration and production of oxidants are the most common targets of the drugs investigated and could be part of their mode of action.

EFFECTS OF LIPOXYGENASE-DERIVED EICOSANOIDS ON FIBROBLASTS: CHEMOTACTIC ACTIVITY AND REGULATION OF COLLAGEN SYNTHESIS.
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Metabolism of fibroblasts (fb) plays an important role in wound healing and can be modulated by other cell types. In a search for soluble molecular signals produced by these cells, we investigated the effects of eicosanoids on fb function. Chemotactic activity of fb was assessed in a blind well Boyden chamber with various eicosanoids as chemoattractants. Fb proliferation was measured by cell counts, and their biosynthetic capacities by 3H-proline labelling. For measuring the synthesis of collagen and non-collagenous proteins, the newly synthesized material was dialyzed, hydrolyzed and analyzed on an automated amino acid analyzer.
LTB4, 5S- and 12S-HETE were found to be strongly chemotactic for fibroblasts. Structure-activity analysis revealed that the position of hydroxylation on C5 and C12 was essential for chemotactic activity. Preincubation of cells with 12S-HETE reduced the chemotactic response to 5S-HETE and LTB4, indicating that these eicosanoids utilize the same cell surface receptor, whereas chemotaxis to other chemoattractants such as fibronectin and PDGF was unaffected by 12-HETE. Total protein synthesis was not influenced by any of the eicosanoids tested, however, a specific decrease of collagen synthesis was noted in the presence of 12S-HETE.
These results suggest a possible role of eicosanoids in the regulation of fb metabolism during chronic inflammation and in fibrotic processes. Of particular interest is the activity of 12-HETE, the main product of arachidonic acid in epidermis with unknown function, which may serve as a molecular signal between epidermis and dermis.

ARACHIDONIC ACID INDUCED EAR OEDEMA IN THREE STRAINS OF RATS AND MICE : A COMPARATIVE STUDY OF ANTI-INFLAMMATORY DRUGS.
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Topical application of arachidonic acid (AA) to the mouse ear induces an inflammatory response associated with the formation of prostaglandins and leucotrienes (CHANG et al., 1986, Inflammation 10, 205-214).
We now report a comparison of the inhibitory responses of three strains of rats (OFA, WISTAR, Hairless) and mice (CD1, C3H, HMR1) to a variety of known anti-inflammatory agents. Oedema responses were measured either at 1 h or 2 h post topical arachidonic acid application, which had previously been found to provoke maximum oedema formation in mice and rats respectively.
Indomethacin, BW755, 5,8,11,14-ETYA were active in all the strains of both rats and mice. Naproxen was less active in mice. Hydrocortisone was inactive in all of the animals tested. Whilst both Betamethasone-17-valerate and Nordihydroguaiaretic Acid were inactive in the different rat strains they were none the less active in the mice strains. The different inhibitory effects obtained for the same anti-inflammatory agent in different animals and strains of animals is probably attributable to a variety of reasons including different end organ effects and pharmacokinetic influences.
These results confirm the need of careful choice in the selection of suitable animals and strains when developping animal models for topically applied drugs.

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